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**WO 2006/074279 A1**

(54) Title: GLYCOCONJUGATION USING SACCHARYL FRAGMENTS

(57) **Abstract:** The present invention provides conjugates between a substrate, e.g., peptide, glycopeptide, lipid, etc., and a modified saccharyl fragment bearing a modifying group such as a water-soluble polymer, therapeutic moiety or a biomolecule. The conjugates are linked via the enzymatic conversion of the activated modified saccharyl fragment into a glycosyl linking group that is interposed between and covalently attached to the substrate and the modifying group. The conjugates are formed from substrates by the action of a sugar transferring enzyme, e.g., a glycosyltransferase. For example, when the substrate is a peptide, the enzyme conjugates a modified saccharyl fragment moiety onto either an amino acid or glycosyl residue of the peptide. Also provided are pharmaceutical formulations that include the conjugates. Methods for preparing the conjugates are also within the scope of the invention.

## GLYCOCONJUGATION USING SACCHARYL FRAGMENTS

### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is related to U.S. Provisional Patent Application  
5 60/641,956, filed January 6, 2005, which is incorporated by reference in its entirety for all purposes.

### BACKGROUND OF THE INVENTION

#### Field of the Invention

[0002] The present invention relates to conjugates formed between a biologically relevant  
10 substrate (e.g., a glycosylated or non-glycosylated peptide or lipid) and a saccharyl fragment that includes a modifying group (“modified fragment”). The substrate and modified fragment are linked through an enzymatically formed bond between the modified fragment and an acceptor moiety on the substrate.

#### Background

15 [0003] The administration of glycosylated and non-glycosylated therapeutic agents for engendering a particular physiological response is well known in the medicinal arts. For example, both purified and recombinant hGH are used for treating conditions and diseases due to hGH deficiency, e.g., dwarfism in children. Interferon has known antiviral activity and granulocyte colony stimulating factor stimulates the production of white blood cells.

20 [0004] A principal factor that has limited the use of therapeutic peptides is the difficulty inherent in engineering an expression system to express a peptide having the glycosylation pattern of the wild-type peptide. Improperly or incompletely glycosylated peptides can be immunogenic; in a patient, an immunogenic response to an administered peptide can neutralize the peptide and/or lead to the development of an allergic response in the patient.

25 Other deficiencies of recombinantly produced glycopeptides include suboptimal potency and rapid clearance rates. The problems inherent in peptide therapeutics are recognized in the art, and various methods of eliminating the problems have been investigated.

[0005] Post-expression *in vitro* modification of peptides is an attractive strategy to remedy the deficiencies of methods that rely on controlling glycosylation by engineering expression

systems; including both modification of glycan structures or introduction of glycans at novel sites. A comprehensive toolbox of recombinant eukaryotic glycosyltransferases is becoming available, making *in vitro* enzymatic synthesis of mammalian glycoconjugates with custom designed glycosylation patterns and glycosyl structures possible. See, for example, U.S.

5 Patent No. 5,876,980; 6,030,815; 5,728,554; 5,922,577; and WO/9831826; US2003180835; and WO 03/031464.

[0006] Enzyme-based syntheses have the advantages of regioselectivity and stereoselectivity. Moreover, enzymatic syntheses are performed using unprotected substrates. Two principal classes of enzymes are used in the synthesis of carbohydrates,  
10 glycosyltransferases (*e.g.*, sialyltransferases, oligosaccharyltransferases, N-acetylglucosaminyltransferases), and glycosidases. The glycosidases are further classified as exoglycosidases (*e.g.*,  $\beta$ -mannosidase,  $\beta$ -glucosidase), and endoglycosidases (*e.g.*, Endo-A, Endo-M). Each of these classes of enzymes has been successfully used synthetically to prepare carbohydrates. For a general review, *see*, Crout *et al.*, *Curr. Opin. Chem. Biol.* **2**: 98-  
15 111 (1998).

[0007] Glycosyltransferases modify the oligosaccharide structures on glycopeptides. Glycosyltransferases are effective for producing specific products with good stereochemical and regiochemical control. Glycosyltransferases have been used to prepare oligosaccharides and to modify terminal N- and O-linked carbohydrate structures, particularly on  
20 glycopeptides produced in mammalian cells. For example, the terminal oligosaccharides of glycopeptides have been completely sialylated and/or fucosylated to provide more consistent sugar structures, which improves glycopeptide pharmacodynamics and a variety of other biological properties. For example,  $\beta$ -1,4-galactosyltransferase was used to synthesize lactosamine, an illustration of the utility of glycosyltransferases in the synthesis of carbohydrates (*see, e.g.*, Wong *et al.*, *J. Org. Chem.* **47**: 5416-5418 (1982)). Moreover,  
25 numerous synthetic procedures have made use of  $\alpha$ -sialyltransferases to transfer sialic acid from cytidine-5'-monophospho-N-acetylneurameric acid to the 3-OH or 6-OH of galactose (*see, e.g.*, Kevin *et al.*, *Chem. Eur. J.* **2**: 1359-1362 (1996)). Fucosyltransferases are used in synthetic pathways to transfer a fucose unit from guanosine-5'-diphosphofucose to a specific  
30 hydroxyl of a saccharide acceptor. For example, Ichikawa prepared sialyl Lewis-X by a method that involves the fucosylation of sialylated lactosamine with a cloned fucosyltransferase (Ichikawa *et al.*, *J. Am. Chem. Soc.* **114**: 9283-9298 (1992)). For a

discussion of recent advances in glycoconjugate synthesis for therapeutic use *see, Koeller et al., Nature Biotechnology* **18**: 835-841 (2000). See also, U.S. Patent No. 5,876,980; 6,030,815; 5,728,554; 5,922,577; and WO/9831826.

[0008] Glycosidases can also be used to prepare saccharides. Glycosidases normally catalyze the hydrolysis of a glycosidic bond. Under appropriate conditions, however, they can be used to form this linkage. Most glycosidases used for carbohydrate synthesis are exoglycosidases; the glycosyl transfer occurs at the non-reducing terminus of the substrate. The glycosidase takes up a glycosyl donor in a glycosyl-enzyme intermediate that is either intercepted by water to give the hydrolysis product, or by an acceptor, to give a new glycoside or oligosaccharide. An exemplary pathway using an exoglycosidase is the synthesis of the core trisaccharide of all N-linked glycopeptides, including the difficult  $\beta$ -mannoside linkage, which was formed by the action of  $\beta$ -mannosidase (Singh *et al.*, *Chem. Commun.* 993-994 (1996)).

[0009] In another exemplary application of the use of a glycosidase to form a glycosidic linkage, a mutant glycosidase was prepared in which the normal nucleophilic amino acid within the active site is changed to a non-nucleophilic amino acid. The mutant enzymes do not hydrolyze glycosidic linkages, but can still form them. The mutant glycosidases are used to prepare oligosaccharides using an  $\alpha$ -glycosyl fluoride donor and a glycoside acceptor molecule (Withers *et al.*, U.S. Patent No. 5,716,812). Although the mutant glycosidases are useful for forming free oligosaccharides, it has yet to be demonstrated that such enzymes are capable of appending glycosyl donors onto glycosylated or non-glycosylated peptides, nor have these enzymes been used with unactivated glycosyl donors.

[0010] Although their use is less common than that of the exoglycosidases, endoglycosidases are also utilized to prepare carbohydrates. Methods based on the use of endoglycosidases have the advantage that an oligosaccharide, rather than a monosaccharide, is transferred. Oligosaccharide fragments have been added to substrates using *endo*- $\beta$ -N-acetylglucosamines such as *endo*-F, *endo*-M (Wang *et al.*, *Tetrahedron Lett.* **37**: 1975-1978); and Haneda *et al.*, *Carbohydr. Res.* **292**: 61-70 (1996)).

[0011] In addition to their use in preparing carbohydrates, the enzymes discussed above are applied to the synthesis of glycopeptides. The synthesis of a homogeneous glycoform of ribonuclease B has been published (Witte K. *et al.*, *J. Am. Chem. Soc.* **119**: 2114-2118

(1997)). The high mannose core of ribonuclease B was cleaved by treating the glycopeptide with endoglycosidase H. The cleavage occurred specifically between the two core GlcNAc residues. The tetrasaccharide sialyl Lewis X was then enzymatically rebuilt on the remaining GlcNAc anchor site on the now homogeneous protein by the sequential use of  $\beta$ -1,4-galactosyltransferase,  $\alpha$ -2,3-sialyltransferase and  $\alpha$ -1,3-fucosyltransferase V. Each enzymatically catalyzed step proceeded in excellent yield.

[0012] Methods combining both chemical and enzymatic synthetic elements are also known. For example, Yamamoto and coworkers (*Carbohydr. Res.* **305**: 415-422 (1998)) reported the chemoenzymatic synthesis of the glycopeptide, glycosylated Peptide T, using an endoglycosidase. The N-acetylglucosaminyl peptide was synthesized by purely chemical means. The peptide was subsequently enzymatically elaborated with the oligosaccharide of human transferrin glycopeptide. The saccharide portion was added to the peptide by treating it with an endo- $\beta$ -N-acetylglucosaminidase. The resulting glycosylated peptide was highly stable and resistant to proteolysis when compared to the peptide T and N-acetylglucosaminyl peptide T.

[0013] The use of glycosyltransferases to modify peptide structure with reporter groups has been explored. For example, Brossmer *et al.* (U.S. Patent No. 5,405,753) discloses the formation of a fluorescent-labeled cytidine monophosphate ("CMP") derivative of sialic acid and the use of the fluorescent glycoside in an assay for sialyl transferase activity and for the fluorescent labeling of cell surfaces, glycoproteins and gangliosides. Gross *et al.* (*Analyt. Biochem.* **186**: 127 (1990)) describe a similar assay. Bean *et al.* (U.S. Patent No. 5,432,059) discloses an assay for glycosylation deficiency disorders utilizing reglycosylation of a deficiently glycosylated protein. The deficient protein is reglycosylated with a fluorescent-labeled CMP glycoside. Each of the fluorescent sialic acid derivatives is substituted with the fluorescent moiety at either the 9-position or at the amine that is normally acetylated in sialic acid. The methods using the fluorescent sialic acid derivatives are assays for the presence of glycosyltransferases or for non-glycosylated or improperly glycosylated glycoproteins. The assays are conducted on small amounts of enzyme or glycoprotein in a sample of biological origin. The enzymatic derivatization of a glycosylated or non-glycosylated peptide on a preparative or industrial scale using a modified sialic acid has not been disclosed or suggested.

[0014] Considerable effort has also been directed towards the modification of cell surfaces by altering glycosyl residues presented by those surfaces. For example, Fukuda and coworkers have developed a method for attaching glycosides of defined structure onto cell surfaces. The method exploits the relaxed substrate specificity of a fucosyltransferase that can transfer fucose and fucose analogs bearing diverse glycosyl substrates (Tsuboi *et al.*, *J. Biol. Chem.* **271**: 27213 (1996)).

[0015] The methods of modifying cell surfaces have not been applied in the absence of a cell to modify a glycosylated or non-glycosylated peptide. Moreover, the methods of cell surface modification are not utilized for the enzymatic incorporation preformed modified glycosyl donor moiety into a peptide. Moreover, none of the cell surface modification methods are practical for producing glycosyl-modified peptides on an industrial scale.

[0016] Enzymatic methods have also been used to activate glycosyl residues on a glycopeptide towards subsequent chemical elaboration. The glycosyl residues are typically activated using galactose oxidase, which converts a terminal galactose residue to the corresponding aldehyde. The aldehyde is subsequently coupled to an amine-containing modifying group. For example, Casares *et al.* (*Nature Biotech.* **19**: 142 (2001)) have attached doxorubicin to the oxidized galactose residues of a recombinant MHCII-peptide chimera.

[0017] In addition to manipulating the structure of glycosyl groups on polypeptides, interest has developed in preparing glycopeptides that are modified with one or more non-saccharide modifying group, such as a water-soluble polymer. Poly(ethyleneglycol) (“PEG”) is an exemplary polymer that has been conjugated to polypeptides. The use of PEG to derivatize peptide therapeutics has been demonstrated to reduce the immunogenicity of the peptides. For example, U.S. Pat. No. 4,179,337 (Davis *et al.*) discloses non-immunogenic polypeptides, such as enzymes and peptide hormones coupled to polyethylene glycol (PEG) or polypropylene glycol. Between 10 and 100 moles of polymer are used per mole polypeptide. Although the *in vivo* clearance time of the conjugate is prolonged relative to that of the polypeptide, only about 15% of the physiological activity is maintained. Thus, the prolonged circulation half-life is counterbalanced by the dramatic reduction in peptide potency.

[0018] The loss of peptide activity is directly attributable to the non-selective nature of the chemistries utilized to conjugate the water-soluble polymer. The principal mode of attachment of PEG, and its derivatives, to peptides is a non-specific bonding through a

peptide amino acid residue. For example, U.S. Patent No. 4,088,538 discloses an enzymatically active polymer-enzyme conjugate of an enzyme covalently bound to PEG. Similarly, U.S. Patent No. 4,496,689 discloses a covalently attached complex of  $\alpha$ -1 proteinase inhibitor with a polymer such as PEG or methoxypoly(ethyleneglycol) (“(m-)PEG”). Abuchowski *et al.* (*J. Biol. Chem.* **252**: 3578 (1977)) discloses the covalent attachment of (m-) PEG to an amine group of bovine serum albumin. U.S. Patent No. 4,414,147 discloses a method of rendering interferon less hydrophobic by conjugating it to an anhydride of a dicarboxylic acid, such as poly(ethylene succinic anhydride). PCT WO 87/00056 discloses conjugation of PEG and poly(oxyethylated) polyols to such proteins as 10 interferon- $\beta$ , interleukin-2 and immunotoxins. EP 154,316 discloses and claims chemically modified lymphokines, such as IL-2 containing PEG bonded directly to at least one primary amino group of the lymphokine. U.S. Patent No. 4,055,635 discloses pharmaceutical compositions of a water-soluble complex of a proteolytic enzyme linked covalently to a polymeric substance such as a polysaccharide.

15 [0019] Another mode of attaching PEG to peptides is through the non-specific oxidation of glycosyl residues on a glycopeptide. The oxidized sugar is utilized as a locus for attaching a PEG moiety to the peptide. For example M'Timkulu (WO 94/05332) discloses the use of an amino-PEG to add PEG to a glycoprotein. The glycosyl moieties are randomly oxidized to the corresponding aldehydes, which are subsequently coupled to the amino-PEG.

20 [0020] In each of the methods described above, poly(ethyleneglycol) is added in a random, non-specific manner to reactive residues on a peptide backbone. For the production of therapeutic peptides, it is clearly desirable to utilize a derivatization strategy that results in the formation of a specifically labeled, readily characterizable, essentially homogeneous product. A promising route to preparing specifically labeled peptides is through the use of enzymes, 25 such as glycosyltransferases to append a modified sugar moiety onto a peptide.

[0021] Glycosyl residues have also been modified to bear ketone groups. For example, Mahal and co-workers (*Science* **276**: 1125 (1997)) have prepared N-levulinoyl mannosamine (“ManLev”), which has a ketone functionality at the position normally occupied by the acetyl group in the natural substrate. Cells were treated with the ManLev, thereby incorporating a 30 ketone group onto the cell surface. See, also Saxon *et al.*, *Science* **287**: 2007 (2000); Hang *et al.*, *J. Am. Chem. Soc.* **123**: 1242 (2001); Yarema *et al.*, *J. Biol. Chem.* **273**: 31168 (1998); and Charter *et al.*, *Glycobiology* **10**: 1049 (2000).

[0022] In addition to an industrially relevant method that utilizes the enzymatic conjugation to specifically conjugate a modified sugar to a peptide or glycopeptide, a method for controlling and manipulating the position of glycosylation on a glycopeptide would be highly desirable.

5 [0023] Carbohydrates are attached to glycopeptides in several ways of which N-linked to asparagine and mucin-type O-linked to serine and threonine are the most relevant for recombinant glycoprotein therapeutics. A determining factor for initiation of glycosylation of a protein is the primary sequence context, although clearly other factors including protein region and conformation play roles. N-linked glycosylation occurs at the consensus sequence  
10 NXS/T, where X can be any amino acid but proline.

[0024] O-linked glycosylation is initiated by a family of about 20 homologous enzymes termed UDP-GalNAc: polypeptide *N*-acetylgalactosaminyltransferases (GalNAc-transferases). O-linked glycosylation does not appear to be ruled by one simple consensus sequence, although studies of the GalNAc-transferase enzymes that initiate O-linked  
15 glycosylation clearly supports the notion that their acceptor specificities are driven by primary sequence contexts. Each of these enzymes transfer a single monosaccharide GalNAc to serine and threonine residues, but they transfer to different peptide sequences although they show a large degree of overlap in functions. It is envisioned that the substrate specificity of each GalNAc-transferase is ruled primarily by a linear short acceptor consensus sequence.

20 [0025] Recently, a method of producing an ester linked carbohydrate-peptide conjugate was described by Davis (WO 03/014371, published Feb. 20, 2003). In this publication, a vinyl ester amino acid group was reacted with a carbohydrate acyl acceptor in the presence of an enzyme such as a protease (such as a serine protease), lipase, esterase or acylase. At this time, however, no other substrates, e.g., glycopeptides, glycolipids, are known to conjugate  
25 with carbohydrate acyl acceptors under these conditions.

[0026] The present invention answers the need for modified therapeutic species in which a modified glycosyl moiety is conjugated onto N- or O-linked glycosylation sites of the peptides and other bioactive species, e.g., glycolipids, sphingosines, ceramides, etc. The invention provides a route to new therapeutic conjugates and addresses the need for more  
30 stable and therapeutically effective species. Moreover, despite the efforts directed toward the enzymatic elaboration of saccharide structures, there remains still a need for alternative industrially practical methods for the modification of therapeutic agents, e.g., peptides,

glycopeptides and lipids with modifying groups such as water-soluble polymers, therapeutic moieties, biomolecules and the like. Of particular interest are methods in which the modified peptide has improved properties, which enhance its use as a therapeutic or diagnostic agent. The present invention fulfills these and other needs.

5

## BRIEF SUMMARY OF THE INVENTION

[0027] Glycotherapeutics (e.g., glycopeptides and glycolipids) present a challenging target for recombinant production of therapeutics. For example, specific carbohydrate moieties are often indispensable for the function and favorable pharmacokinetic properties of glycopeptide therapeutics; however, many of the most robust expression systems produce glycopeptides

10 with non-human glycosylation patterns. Incorrect glycosylation can produce a peptide that is inactive, aggregated, antigenic and/or has unfavorable pharmacokinetics. Accordingly, considerable efforts are expended to develop recombinant expression cell systems capable of producing glycoproteins with biologically appropriate carbohydrate structures. This approach is hampered by numerous shortcomings, including cost, and heterogeneity and  
15 limitations in glycan structures.

[0028] Post-expression, *in vitro* glyco-modification of glycotherapeutics, e.g., glycopeptides, is an attractive strategy to remedy the deficiencies of methods that rely on controlling glycosylation by engineering expression systems; including both modification of glycan structures or introduction of glycans at novel sites. A comprehensive toolbox of

20 recombinant eukaryotic glycosyltransferases is becoming available, making *in vitro* enzymatic synthesis of glycoconjugates with custom designed glycosylation patterns and glycosyl structures possible. See, for example, U.S. Patent Nos. 5,876,980; 6,030,815; 5,728,554; and 5,922,577; and WO 98/31826; US03/180835; and WO 03/031464.

[0029] *In vitro* glycosylation offers a number of advantages compared to recombinant expression of glycoproteins of which custom design and higher degree of homogeneity of the glycosyl moiety are examples. Moreover, combining bacterial expression of glycotherapeutics with *in vitro* modification (or placement) of the glycosyl residue offers numerous advantages over traditional recombinant expression technology including reduced potential exposure to adventitious agents, increased homogeneity of product, and cost reduction.

[0030] Ideally, conjugates of therapeutic species, such as peptides and lipids, are obtained using methods that provide the conjugates in a reproducible and predictable manner. Moreover, in forming the conjugates it is generally preferred that the site of conjugation between the therapeutic species and the modifying group is selected such that its modification does not adversely affect advantageous properties of the therapeutic species, e.g. activity, specificity, low antigenicity, low toxicity, etc.

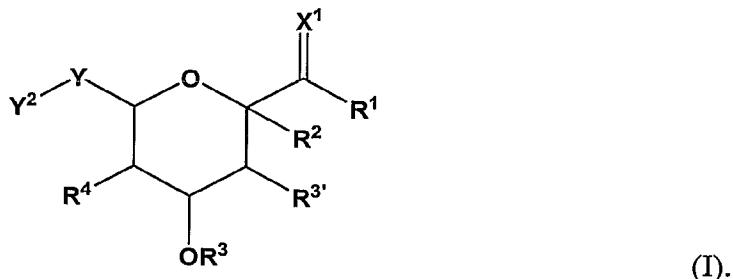
[0031] The present invention provides a method of forming conjugates between a glycosyl residue, amino acid or aglycone moiety of a selected substrate (e.g., (glyco)peptide, (glyco)lipid, etc.) and a modifying group, such as a water-soluble- or water-insoluble- polymer, a therapeutic moiety or a diagnostic agent. The invention exploits the recognition that saccharides, e.g., sialic acid, can be oxidized in a predictable and reproducible fashion , converting a primary or secondary hydroxyl moiety to an aldehyde or a ketone. The carbonyl moiety is readily modified with an amine-containing modifying group, affording a Schiff base, which is reduced to the corresponding amine modified saccharyl fragment. The fragment is recognized as a substrate by one or more enzyme capable of transferring a glycosyl moiety onto a substrate.

[0032] In an exemplary embodiment, the modified saccharyl fragment is a substrate for an enzyme that transfers a glycosyl donor moiety to a glycosyl acceptor. In an exemplary embodiment, the enzyme is a transferase, e.g., a sialyltransferase, which utilizes the modified fragment as a saccharyl donor in an enzymatically-mediated glycosylation reaction. In another embodiment, the enzyme is a mutant of a degradative enzyme, such as an exo- or endoglycosidase, amidase, etc.

[0033] In another embodiment, the modified saccharyl fragment is coupled to an intact saccharide residue. For example, coupling Sia<sup>\*</sup>-(modifying group) to galactose affords, Gal-Sia<sup>\*</sup>-(modifying group), which serves as a glycosyl donor that is added to a substrate, e.g, peptide, lipid, aglycone, etc.

[0034] The present invention is exemplified by reference to modified saccharyl fragments in which the side chain of a sialic acid is oxidized and the resulting carbonyl moiety (aldehyde) is converted to an amine by reductive amination with ammonia or an amine-containing modifying group. Those of skill will appreciate that saccharides, as a group, possess a rich oxidation chemistry that is readily exploited in variations on the exemplification of the invention presented herein.

**[0035]** In an exemplary aspect, the present invention provides a conjugate of a bioactive species, e.g., a peptide, nucleotide, activating moiety, carbohydrate, lipid (e.g., ceramide or sphingosine) that includes a subunit according to Formula I:



5    **[0036]** In Formula I, the symbol  $\text{X}^1$  represents substituted or unsubstituted alkyl, O or  $\text{NR}^8$ .  $\text{R}^8$  is a member selected from H, OH, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. Appropriate  $\text{R}^1$  groups are selected from  $\text{OR}^9$ ,  $\text{NR}^9\text{R}^{10}$ , substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. The symbols  $\text{R}^9$  and  $\text{R}^{10}$  independently represent H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl and  $\text{C}(\text{O})\text{R}^{11}$ .  $\text{R}^{11}$  is a group such as substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl.

10    **[0037]** The symbol  $\text{R}^2$  is a member selected from an is a member selected from a nucleotide, an activating moiety, an amino acid residue of a peptide, a carbohydrate moiety attached to an amino acid residue of a peptide, a carbohydrate moiety attached to an amino acid residue of a peptide through a linker and a carbohydrate moiety attached to an amino acid residue of a peptide through a linker comprising at least a second carbohydrate moiety. Exemplary linkers include one or more additional carbohydrate moieties in addition to that of  $\text{R}^2$ .  $\text{R}^3$  is a member selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. The symbols  $\text{R}^4$  and  $\text{R}^{3'}$  independently represent H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, OH,  $\text{OR}^4$  and  $\text{NHC}(\text{O})\text{R}^{12}$ .  $\text{R}^4$  is a member selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl.  $\text{R}^{12}$  is a member selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl and  $\text{NR}^{13}\text{R}^{14}$ , in which  $\text{R}^{13}$  and  $\text{R}^{14}$  are members independently selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl.

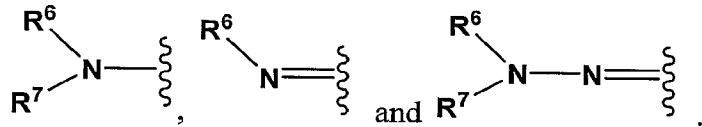
15    **[0037]** The symbol  $\text{R}^2$  is a member selected from an is a member selected from a nucleotide, an activating moiety, an amino acid residue of a peptide, a carbohydrate moiety attached to an amino acid residue of a peptide, a carbohydrate moiety attached to an amino acid residue of a peptide through a linker and a carbohydrate moiety attached to an amino acid residue of a peptide through a linker comprising at least a second carbohydrate moiety. Exemplary linkers include one or more additional carbohydrate moieties in addition to that of  $\text{R}^2$ .  $\text{R}^3$  is a member selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. The symbols  $\text{R}^4$  and  $\text{R}^{3'}$  independently represent H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, OH,  $\text{OR}^4$  and  $\text{NHC}(\text{O})\text{R}^{12}$ .  $\text{R}^4$  is a member selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl.  $\text{R}^{12}$  is a member selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl and  $\text{NR}^{13}\text{R}^{14}$ , in which  $\text{R}^{13}$  and  $\text{R}^{14}$  are members independently selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl.

20    **[0037]** The symbol  $\text{R}^2$  is a member selected from an is a member selected from a nucleotide, an activating moiety, an amino acid residue of a peptide, a carbohydrate moiety attached to an amino acid residue of a peptide, a carbohydrate moiety attached to an amino acid residue of a peptide through a linker and a carbohydrate moiety attached to an amino acid residue of a peptide through a linker comprising at least a second carbohydrate moiety. Exemplary linkers include one or more additional carbohydrate moieties in addition to that of  $\text{R}^2$ .  $\text{R}^3$  is a member selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. The symbols  $\text{R}^4$  and  $\text{R}^{3'}$  independently represent H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, OH,  $\text{OR}^4$  and  $\text{NHC}(\text{O})\text{R}^{12}$ .  $\text{R}^4$  is a member selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl.  $\text{R}^{12}$  is a member selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl and  $\text{NR}^{13}\text{R}^{14}$ , in which  $\text{R}^{13}$  and  $\text{R}^{14}$  are members independently selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl.

25    **[0037]** The symbol  $\text{R}^2$  is a member selected from an is a member selected from a nucleotide, an activating moiety, an amino acid residue of a peptide, a carbohydrate moiety attached to an amino acid residue of a peptide, a carbohydrate moiety attached to an amino acid residue of a peptide through a linker and a carbohydrate moiety attached to an amino acid residue of a peptide through a linker comprising at least a second carbohydrate moiety. Exemplary linkers include one or more additional carbohydrate moieties in addition to that of  $\text{R}^2$ .  $\text{R}^3$  is a member selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. The symbols  $\text{R}^4$  and  $\text{R}^{3'}$  independently represent H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, OH,  $\text{OR}^4$  and  $\text{NHC}(\text{O})\text{R}^{12}$ .  $\text{R}^4$  is a member selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl.  $\text{R}^{12}$  is a member selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl and  $\text{NR}^{13}\text{R}^{14}$ , in which  $\text{R}^{13}$  and  $\text{R}^{14}$  are members independently selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl.

[0038] Y is the residue of the sialic acid side chain remaining following oxidation to a carbonyl and subsequent reaction of the carbonyl moiety with a nucleophilic group, alternatively followed by additional modifications. Exemplary groups for Y include CH<sub>2</sub>, CH(OH)CH<sub>2</sub>, CH(OH)CH(OH)CH<sub>2</sub> when the oxidation leads to formation of an aldehyde 5 that is subsequently reductively aminated. When the aldehyde is converted to an imine species or is reacted with a phosphorus ylide, Y is typically CH, CH(OH)CH or CH(OH)CH(OH)CH. When the aldehyde is reacted with a Grignard or lithium reagent, exemplary Y groups include CH(OH), CH(OH)CH(OH), CH(OH)CH(OH)CH(OH) or an elimination product thereof, e.g., dehydration product.

[0039] The symbol Y<sup>2</sup> represents groups formed by addition to the carbonyl moiety of the fragment. Y<sup>2</sup> includes at least one modifying group e.g., biomolecule, therapeutic moiety, diagnostic moiety, and a polymeric modifying group, as exemplified by the term R<sup>6a</sup>. Exemplary identities for Y<sup>2</sup> include substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl (e.g., formed by Wittig, Grignard or other appropriate chemistries), 15 R<sup>6</sup>, and nitrogen-containing species, e.g.,



In an exemplary embodiment, Y<sup>2</sup> is a member selected from substituted alkyl, substituted or unsubstituted heteroalkyl, R<sup>6</sup>, and nitrogen-containing species. R<sup>6</sup> and R<sup>7</sup> are independently H, C(O)R<sup>6b</sup> or -L<sup>a</sup>-R<sup>6b</sup> wherein R<sup>6b</sup> is H or R<sup>6a</sup> and L<sup>a</sup> is selected from a bond and a linker 20 group.

[0040] When Y<sup>2</sup> is substituted or unsubstituted alkyl, e.g., an alkene species formed by a Wittig reaction, or saturated species formed by Grignard or lithium chemistries, Y<sup>2</sup> includes at least one modifying group (water-soluble or -insoluble polymer) as exemplified by the term R<sup>6a</sup>.

[0041] As discussed herein, R<sup>6a</sup> can be a polymeric modifying group. Preferred polymeric modifying groups include PEG. The PEG of use in the conjugates of the invention can be linear or branched. An exemplary precursor of use to form the branched PEG containing peptide conjugates according to this embodiment of the invention has the formula:

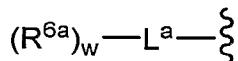


The branched polymer species according to this formula are essentially pure polymeric modifying groups.  $X^{3'}$  is a moiety that includes an ionizable (*e.g.*, OH, COOH, H<sub>2</sub>PO<sub>4</sub>, HSO<sub>3</sub>, NH<sub>2</sub>, and salts thereof, etc.) or other reactive functional group, *e.g.*, *infra*. C is carbon.

5       $X^5$ ,  $R^{16}$  and  $R^{17}$  are independently selected from non-reactive groups (*e.g.*, H, unsubstituted alkyl, unsubstituted heteroalkyl) and polymeric arms (*e.g.*, PEG).  $X^2$  and  $X^4$  are linkage fragments that are preferably essentially non-reactive under physiological conditions and that may be the same or different. An exemplary linker includes neither aromatic nor ester moieties. Alternatively, these linkages can include one or more moiety that is designed to

10     degrade under physiologically relevant conditions, *e.g.*, esters, disulfides, etc.  $X^2$  and  $X^4$  join polymeric arms  $R^{16}$  and  $R^{17}$  to C. When  $X^{3'}$  is reacted with a reactive functional group of complementary reactivity on a linker, sugar or linker-sugar cassette,  $X^{3'}$  is converted to a component of linkage fragment  $X^3$ .

[0042]     In an exemplary embodiment, the polymeric modifying group is bound to the glycosyl linking group, through a linker,  $L^a$ , in which case the residues  $R^6$  and  $R^7$  are independently as shown below:



$R^{6a}$  is the polymeric modifying group and  $L^a$  is selected from a bond and a linking group. The index w represents an integer selected from 1-6, preferably 1-3 and more preferably 1-2.

20     Exemplary linking groups include substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl moieties. An exemplary component of the linker group is an acyl moiety. Another exemplary linking group is an amino acid (*e.g.*, cysteine, serine, lysine, and short oligopeptides, *e.g.*, Lys-Lys, Lys-Lys-Lys, Cys-Lys, Ser-Lys, etc.).

[0043]     When  $L^a$  is a bond, it is formed by reaction of a reactive functional group on a precursor of  $R^{6a}$  and a reactive functional group of complementary reactivity on a precursor of the glycosyl linking group. When  $L^a$  is a non-zero order linking group, L can be in place on the glycosyl moiety prior to reaction with the  $R^{6a}$  precursor. Alternatively, the precursors of  $R^{6a}$  and  $L^a$  can be incorporated into a preformed cassette that is subsequently attached to the glycosyl moiety. As set forth herein, the selection and preparation of precursors with

appropriate reactive functional groups is within the ability of those skilled in the art. Moreover, coupling of the precursors proceeds by chemistry that is well understood in the art.

**[0044]** In another aspect, the invention provides an activated glycosyl linking group that is of use in the methods of the invention. In an exemplary embodiment, according to this

5 aspect, the glycosyl linking group has a structure according to Formula I in which R<sup>2</sup> is a nucleotide, forming a nucleotide sugar in which the sugar moiety is, or includes, the saccharyl fragment. R<sup>2</sup> can also be a leaving group (activating group), such as a halogen, sulfonate ester and the like.

**[0045]** In a third aspect, the invention provides a peptide or lipid conjugate having a  
10 population of water-soluble polymer moieties covalently bound thereto through a glycosyl linking group that includes a moiety according to Formula I. In the conjugate of the invention, essentially each member of the population is bound via a glycosyl linking group, that includes a subunit according to Formula I, to an amino acid or glycosyl residue of the peptide, and each amino acid or glycosyl residue to which the linking group is bound has the  
15 same structure.

**[0046]** In a fourth aspect, the invention provides a method of forming a covalent conjugate between a polymer, e.g., water-soluble polymer, and saccharyl acceptor that is a glycosylated-peptide or -lipid, or a non-glycosylated-peptide or -lipid. The polymer is conjugated to the acceptor via a glycosyl linking group that includes a moiety according to  
20 Formula I. The glycosyl linking group is interposed between, and covalently linked either directly or indirectly to both the acceptor and the polymer. The method includes contacting the acceptor with a mixture containing a modified saccharyl fragment, generally activated as the nucleotide derivative, and an enzyme for which the modified saccharyl fragment is a substrate. The mixture also includes an enzyme that transfers a saccharyl residue, for which  
25 the modified saccharyl fragment is a substrate. The reaction is conducted under conditions appropriate to form the conjugate. See, for example WO03/031464 and related U.S. and PCT applications.

**[0047]** In a fifth aspect, the invention provides a conjugate analogous to those described above, in which the modified saccharyl fragment is derivatized with a therapeutic or  
30 diagnostic moiety. In an exemplary embodiment, the modifying group is a biomolecule, which can be a therapeutic or diagnostic agent.

[0048] In a further aspect, the present invention provides a composition for forming a conjugate between a peptide or lipid and a modified saccharyl fragment. The composition generally includes an activated analogue of the saccharyl fragment set forth in Formula I, an enzyme for which the activated glycosyl linking group is a substrate, and a (glyco)peptide or (glyco)lipid acceptor substrate. The glycosyl linking group has covalently attached thereto a member selected from water-soluble polymers, therapeutic moieties and biomolecules.

[0049] Also provided is a pharmaceutical composition. The composition includes a pharmaceutically acceptable carrier and a conjugate of the invention in admixture with a pharmaceutically acceptable carrier.

10 [0050] Other objects and advantages of the invention will be apparent to those of skill in the art from the detailed description that follows.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0051] FIG. 1 is a table of peptides to which the modified saccharyl fragment can be attached.

15 [0052] FIG. 2 is a table of sialyltransferases of use in practicing the present invention.

#### DETAILED DESCRIPTION OF THE INVENTION AND THE PREFERRED EMBODIMENTS

##### Abbreviations

[0053] Branched or un-branched PEG, poly(ethyleneglycol), including m-PEG, methoxy-  
20 poly(ethylene glycol); branched or unbranched PPG, poly(propyleneglycol); m-PPG,  
methoxy-poly(propylene glycol); Fuc, fucosyl; Gal, galactosyl; GalNAc, N-  
acetylgalactosaminyl; Glc, glucosyl; GlcNAc, N-acetylglucosaminyl; Man, mannosyl;  
ManAc, mannosaminyl acetate; Sia, sialic acid; NeuAc, N-acetylneuraminy; and SA\*-Y,  
25 sialic acid fragment, wherein SA\* is the glycosidic core or ring structure of the molecule and  
Y is part of the modified sialic acid side chain.

##### Definitions

[0054] Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory

procedures in cell culture, molecular genetics, organic chemistry and nucleic acid chemistry and hybridization are those well known and commonly employed in the art. Standard techniques are used for nucleic acid and peptide synthesis. The techniques and procedures are generally performed according to conventional methods in the art and various general

5 references (see generally, Sambrook *et al.* MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., which is incorporated herein by reference), which are provided throughout this document. The nomenclature used herein and the laboratory procedures in analytical chemistry, and organic synthetic described below are those well known and commonly employed in the art.

10 Standard techniques, or modifications thereof, are used for chemical syntheses and chemical analyses.

[0055] The term “alkyl,” by itself or as part of another substituent, means, unless otherwise stated, a straight or branched chain, or cyclic hydrocarbon radical, or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent radicals, having the number of carbon atoms designated (*i.e.* C<sub>1</sub>-C<sub>10</sub> means one to ten carbons). Examples of saturated hydrocarbon radicals include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, cyclohexyl, (cyclohexyl)methyl, cyclopropylmethyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butynyl, and the higher homologs and isomers. The term “alkyl,” unless otherwise noted, is also meant to include those derivatives of alkyl defined in more detail below, such as “heteroalkyl.” Alkyl groups, which are limited to 25 hydrocarbon groups are termed “homoalkyl”.

[0056] The term “alkylene” by itself or as part of another substituent means a divalent radical derived from an alkane, as exemplified, but not limited, by -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-, and further includes those groups described below as “heteroalkylene.” Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer 30 carbon atoms being preferred in the present invention. A “lower alkyl” or “lower alkylene” is a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms.

[0057] The terms “alkoxy,” “alkylamino” and “alkylthio” (or thioalkoxy) are used in their conventional sense, and refer to those alkyl groups attached to the remainder of the molecule via an oxygen atom, an amino group, or a sulfur atom, respectively.

[0058] The term “heteroalkyl,” by itself or in combination with another term, means, unless

5 otherwise stated, a stable straight or branched chain, or cyclic hydrocarbon radical, or combinations thereof, consisting of the stated number of carbon atoms and at least one heteroatom selected from the group consisting of O, N, Si and S, and wherein the nitrogen and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N and S and Si may be placed at any interior position of  
10 the heteroalkyl group or at the position at which the alkyl group is attached to the remainder of the molecule. Examples include, but are not limited to, -CH<sub>2</sub>-CH<sub>2</sub>-O-CH<sub>3</sub>, -CH<sub>2</sub>-CH<sub>2</sub>-NH-CH<sub>3</sub>, -CH<sub>2</sub>-CH<sub>2</sub>-N(CH<sub>3</sub>)-CH<sub>3</sub>, -CH<sub>2</sub>-S-CH<sub>2</sub>-CH<sub>3</sub>, -CH<sub>2</sub>-CH<sub>2</sub>-S(O)-CH<sub>3</sub>, -CH<sub>2</sub>-CH<sub>2</sub>-S(O)<sub>2</sub>-CH<sub>3</sub>, -CH=CH-O-CH<sub>3</sub>, -Si(CH<sub>3</sub>)<sub>3</sub>, -CH<sub>2</sub>-CH=N-OCH<sub>3</sub>, and -CH=CH-N(CH<sub>3</sub>)-CH<sub>3</sub>. Up to two heteroatoms may be consecutive, such as, for example, -CH<sub>2</sub>-NH-OCH<sub>3</sub> and -CH<sub>2</sub>-O-  
15 Si(CH<sub>3</sub>)<sub>3</sub>. Similarly, the term “heteroalkylene” by itself or as part of another substituent means a divalent radical derived from heteroalkyl, as exemplified, but not limited by, -CH<sub>2</sub>-CH<sub>2</sub>-S-CH<sub>2</sub>-CH<sub>2</sub>- and -CH<sub>2</sub>-S-CH<sub>2</sub>-CH<sub>2</sub>-NH-CH<sub>2</sub>-.  
For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini (e.g., alkyleneoxy, alkylenedioxy, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene  
20 linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group is written. For example, the formula -C(O)<sub>2</sub>R'- represents both -C(O)<sub>2</sub>R'- and -R'C(O)<sub>2</sub>-.

[0059] In general, an “acyl substituent” is also selected from the group set forth above. As used herein, the term “acyl substituent” refers to groups attached to, and fulfilling the valence  
25 of a carbonyl carbon that is either directly or indirectly attached to the polycyclic nucleus of the compounds of the present invention.

[0060] The terms “cycloalkyl” and “heterocycloalkyl”, by themselves or in combination with other terms, represent, unless otherwise stated, cyclic versions of “alkyl” and

30 “heteroalkyl”, respectively. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include, but are not

limited to, 1 -(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1 -piperazinyl, 2-piperazinyl, and the like.

**[0061]** The terms “halo” or “halogen,” by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as “haloalkyl,” are meant to include monohaloalkyl and polyhaloalkyl. For example, the term “halo(C<sub>1</sub>-C<sub>4</sub>)alkyl” is mean to include, but not be limited to, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

**[0062]** The term “aryl” means, unless otherwise stated, a polyunsaturated, aromatic, hydrocarbon substituent which can be a single ring or multiple rings (preferably from 1 to 3 rings) which are fused together or linked covalently. The term “heteroaryl” refers to aryl groups (or rings) that contain from one to four heteroatoms selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule through a heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxaliny, 5-quinoxaliny, 3-quinolyl, and 6-quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below.

**[0063]** For brevity, the term “aryl” when used in combination with other terms (*e.g.*, aryloxy, arylthioxy, arylalkyl) includes both aryl and heteroaryl rings as defined above. Thus, the term “arylalkyl” is meant to include those radicals in which an aryl group is attached to an alkyl group (*e.g.*, benzyl, phenethyl, pyridylmethyl and the like) including those alkyl groups in which a carbon atom (*e.g.*, a methylene group) has been replaced by, for example, an oxygen atom (*e.g.*, phenoxyethyl, 2-pyridyloxymethyl, 3-(1-naphthoxypropyl, and the like).

**[0064]** Each of the above terms (*e.g.*, “alkyl,” “heteroalkyl,” “aryl” and “heteroaryl”) include both substituted and unsubstituted forms of the indicated radical. Preferred substituents for each type of radical are provided below.

**[0065]** Substituents for the alkyl, and heteroalkyl radicals (including those groups often

5 referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) are generally referred to as “alkyl substituents” and “heteroalkyl substituents,” respectively, and they can be one or more of a variety of groups selected from, but not limited to: -OR’, =O, =NR’, =N-OR’, -NR’R”, -SR’, -halogen, -SiR’R”R””, -OC(O)R’, -C(O)R’, -CO<sub>2</sub>R’, -CONR’R”, -OC(O)NR’R”, -NR”C(O)R’, -NR’-C(O)NR”R””, -NR”C(O)<sub>2</sub>R’, -NR-C(NR’R”R””)=NR””, -NR-C(NR’R”)=NR””, -S(O)R’, -S(O)<sub>2</sub>R’, -S(O)<sub>2</sub>NR’R”, -NRSO<sub>2</sub>R’, -CN and -NO<sub>2</sub> in a number ranging from zero to (2m’+1), where m’ is the total number of carbon atoms in such radical. R’, R”, R”” and R””” each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, *e.g.*, aryl substituted with 1-3 halogens, substituted or unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R’, R”, R”” and R””” groups when more than one of these groups is present. When R’ and R” are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 5-, 6-, or 7-membered ring. For example, -

10 NR’R” is meant to include, but not be limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term “alkyl” is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (*e.g.*, -CF<sub>3</sub> and -CH<sub>2</sub>CF<sub>3</sub>) and acyl (*e.g.*, -C(O)CH<sub>3</sub>, -C(O)CF<sub>3</sub>, -C(O)CH<sub>2</sub>OCH<sub>3</sub>, and the like).

15

20 **[0066]** Similar to the substituents described for the alkyl radical, the aryl substituents and heteroaryl substituents are generally referred to as “aryl substituents” and “heteroaryl substituents,” respectively and are varied and selected from, for example: halogen, -OR’, =O, =NR’, =N-OR’, -NR’R”, -SR’, -halogen, -SiR’R”R””, -OC(O)R’, -C(O)R’, -CO<sub>2</sub>R’, -CONR’R”, -OC(O)NR’R”, -NR”C(O)R’, -NR’-C(O)NR”R””, -NR”C(O)<sub>2</sub>R’, -NR-C(NR’R”R””)=NR””, -S(O)R’, -S(O)<sub>2</sub>R’, -S(O)<sub>2</sub>NR’R”, -NRSO<sub>2</sub>R’, -CN and -NO<sub>2</sub>, -R’, -N<sub>3</sub>, -CH(Ph)<sub>2</sub>, fluoro(C<sub>1</sub>-C<sub>4</sub>)alkoxy, and fluoro(C<sub>1</sub>-C<sub>4</sub>)alkyl, in a number ranging from zero to the total number of open valences on the aromatic ring system; and where R’, R”, R”” and

25

R<sup>””</sup> are preferably independently selected from hydrogen, (C<sub>1</sub>-C<sub>8</sub>)alkyl and heteroalkyl, unsubstituted aryl and heteroaryl, (unsubstituted aryl)-(C<sub>1</sub>-C<sub>4</sub>)alkyl, and (unsubstituted aryl)oxy-(C<sub>1</sub>-C<sub>4</sub>)alkyl. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R<sup>””</sup> and R<sup>”””</sup> groups when more than one of these groups is present.

[0067] Two of the aryl substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -T-C(O)-(CRR')<sub>q</sub>-U-, wherein T and U are independently -NR-, -O-, -CRR'- or a single bond, and q is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may 10 optionally be replaced with a substituent of the formula -A-(CH<sub>2</sub>)<sub>r</sub>-B-, wherein A and B are independently -CRR'-, -O-, -NR-, -S-, -S(O)-, -S(O)<sub>2</sub>-, -S(O)<sub>2</sub>NR'- or a single bond, and r is an integer of from 1 to 4. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -  
15 (CRR')<sub>s</sub>-X-(CR''R<sup>””</sup>)<sub>d</sub>-, where s and d are independently integers of from 0 to 3, and X is -O-, -NR'-, -S-, -S(O)-, -S(O)<sub>2</sub>-, or -S(O)<sub>2</sub>NR'-. The substituents R, R', R'' and R<sup>””</sup> are preferably independently selected from hydrogen or substituted or unsubstituted (C<sub>1</sub>-C<sub>6</sub>)alkyl.

[0068] As used herein, the term “heteroatom” includes oxygen (O), nitrogen (N), sulfur (S) and silicon (Si).

20 [0069] The term “nucleic acid” or “polynucleotide” refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless  
25 otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, *Nucleic Acid Res.* **19**:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* **260**:2605-2608 (1985); and Rossolini *et al.*, *Mol. Cell. Probes* **8**:91-98

(1994)). The term nucleic acid is used interchangeably with gene, cDNA, and mRNA encoded by a gene.

5 [0070] The term “gene” means the segment of DNA involved in producing a polypeptide chain. It may include regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

10 [0071] The term “amino acid” refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline,  $\gamma$ -carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an  $\alpha$  carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. “Amino acid mimetics” refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but functioning in a manner similar to a naturally occurring amino acid.

15 [0072] Amino acids may be referred to herein by either the commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

20 [0073] “Conservatively modified variants” applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, “conservatively modified variants” refers to those nucleic acids that encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are “silent variations,” which are one species of conservatively modified variations. Every nucleic acid sequence herein that encodes a polypeptide also describes every possible silent variation of

the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule.

Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in  
5 each described sequence.

[0074] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a “conservatively modified variant” where the alteration results in the substitution  
10 of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0075] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a “conservatively modified variant” where the alteration results in the substitution  
15 of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.  
20

[0076] The following eight groups each contain amino acids that are conservative substitutions for one another:

- 1) Alanine (A), Glycine (G);
- 2) Aspartic acid (D), Glutamic acid (E);
- 3) Asparagine (N), Glutamine (Q);
- 4) Arginine (R), Lysine (K);
- 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
- 7) Serine (S), Threonine (T); and
- 8) Cysteine (C), Methionine (M)

(*see, e.g.*, Creighton, *Proteins* (1984)).

[0077] Amino acids may be referred to herein by either the common three-letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0078] The term "mutating" or "mutation," as used in the context of altering the structure or enzymatic activity of a wild-type enzyme, refers to the deletion, insertion, or substitution of any nucleotide or amino acid residue, by chemical, enzymatic, or any other means, in a polynucleotide sequence encoding a that enzyme or the amino acid sequence of a wild-type enzyme, respectively, such that the amino acid sequence of the resulting enzyme is altered at one or more amino acid residues. The site for such an activity-altering mutation may be located anywhere in the enzyme, but is preferably within the active site of the enzyme.

[0079] "Peptide" refers to a polymer in which the monomers are amino acids and are joined together through amide bonds, alternatively referred to as a polypeptide. Additionally, unnatural amino acids, for example,  $\beta$ -alanine, phenylglycine and homoarginine are also included. Amino acids that are not gene-encoded may also be used in the present invention. Furthermore, amino acids that have been modified to include reactive groups, glycosylation sites, polymers, therapeutic moieties, biomolecules and the like may also be used in the invention. All of the amino acids used in the present invention may be either the D - or L - isomer. The L -isomer is generally preferred. In addition, other peptidomimetics are also useful in the present invention. As used herein, "peptide" refers to both glycosylated and unglycosylated peptides. Also included are peptides that are incompletely glycosylated by a system that expresses the peptide. For a general review, *see*, Spatola, A. F., in *CHEMISTRY AND BIOCHEMISTRY OF AMINO ACIDS, PEPTIDES AND PROTEINS*, B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983).

[0080] The term "peptide conjugate," refers to species of the invention in which a peptide is conjugated with an acyl-containing group that is attached to the peptide through a sugar residue.

[0081] The term "sialic acid" refers to any member of a family of nine-carbon carboxylated sugars. The most common member of the sialic acid family is N-acetyl-neuraminic acid (2-keto-5-acetamido-3,5-dideoxy-D-glycero-D-galactonulopyranos-1-onic acid (often

abbreviated as Neu5Ac, NeuAc, or NANA). A second member of the family is N-glycolyl-neuraminic acid (Neu5Gc or NeuGc), in which the N-acetyl group of NeuAc is hydroxylated. A third sialic acid family member is 2-keto-3-deoxy-nonulosonic acid (KDN) (Nadano *et al.* (1986) *J. Biol. Chem.* **261**: 11550-11557; Kanamori *et al.*, *J. Biol. Chem.* **265**: 21811-21819 (1990)). Also included are 9-substituted sialic acids such as a 9-O-C<sub>1</sub>-C<sub>6</sub> acyl-Neu5Ac like 9-O-lactyl-Neu5Ac or 9-O-acetyl-Neu5Ac, 9-deoxy-9-fluoro-Neu5Ac and 9-azido-9-deoxy-Neu5Ac. For review of the sialic acid family, *see, e.g.*, Varki, *Glycobiology* **2**: 25-40 (1992); *Sialic Acids: Chemistry, Metabolism and Function*, R. Schauer, Ed. (Springer-Verlag, New York (1992)). The synthesis and use of sialic acid compounds in a sialylation procedure is disclosed in international application WO 92/16640, published October 1, 1992.

[0082] As used herein, the term “modified saccharyl fragment,” refers to a fragment of a naturally- or non-naturally-occurring carbohydrate that has been modified, typically oxidatively to create a locus for attaching a modifying group. In an exemplary embodiment, the saccharyl fragment is a sialic acid fragment in which the side chain is altered by oxidative degradation. The oxidation produces a carbonyl moiety that is subsequently reductively aminated with an amine analogue of the modifying group. In another exemplary embodiment, the ring structure of the saccharide is linearized by reductive conversion to an alditol (e.g., mannose to mannitol) and derivatized, *e.g.*, at one or more of the primary hydroxyl moieties. Useful, modifying groups include, but are not limited to, water-soluble polymers, water-insoluble polymers, therapeutic moieties, diagnostic moieties, biomolecules and the like.

[0083] The term “water-soluble” refers to moieties that have a detectable degree of solubility in water. Methods to detect and/or quantify water solubility are well known in the art. Exemplary water-soluble polymers include peptides, saccharides, poly(ethers), poly(amines), poly(carboxylic acids) and the like. Peptides can have mixed sequences or be composed of a single amino acid, *e.g.*, poly(lysine), poly(aspartic acid), and poly(glutamic acid). An exemplary polysaccharide is poly(sialic acid). An exemplary poly(ether) is poly(ethylene glycol), *e.g.*, m-PEG. Poly(ethylene imine) is an exemplary polyamine, and poly(acrylic) acid is a representative poly(carboxylic acid).

[0084] The polymer backbone of the water-soluble polymer can be poly(ethylene glycol) (PEG), *e.g.*, m-PEG. However, it should be understood that other related polymers are also suitable for use in the practice of this invention and that the use of the term PEG or

poly(ethylene glycol) is intended to be inclusive and not exclusive in this respect. The term PEG includes poly(ethylene glycol) in any of its forms, including alkoxy PEG, alkyl PEG (e.g., mPEG), difunctional PEG, multiarmed PEG, forked PEG, branched PEG, pendent PEG (i.e. PEG or related polymers having one or more functional groups pendent to the polymer backbone), or PEG with degradable linkages therein.

[0085] The polymer backbone can be linear or branched. Branched polymer backbones are generally known in the art. Typically, a branched polymer has a central branch core moiety and a plurality of linear polymer chains linked to the central branch core. PEG is commonly used in branched forms that can be prepared by addition of ethylene oxide to various polyols, such as glycerol, pentaerythritol and sorbitol. The central branch moiety can also be derived from several amino acids, such as lysine. The branched poly(ethylene glycol) can be represented in general form as R(-PEG-OH)<sub>m</sub>, in which R represents the core moiety, such as glycerol, pentaerythritol, amino acid (e.g., cysteine, serine, di-lysine, tri-lysine, etc.) and *m* represents the number of arms. Multi-armed PEG molecules, such as those described in U.S.

10 Pat. No.s 5,932,462; 5,643,575; European Patent Application 0473,084 A2; WO 96/41813 (and its priority documents), can also be used as the polymer backbone.

[0086] Many other polymers are also suitable for the invention. Polymer backbones that are non-peptidic and water-soluble, with from 2 to about 300 termini, are particularly useful in the invention. Examples of suitable polymers include, but are not limited to, other 20 poly(alkylene glycols), such as poly(propylene glycol) ("PPG"), copolymers of ethylene glycol and propylene glycol and the like, poly(oxyethylated polyol), poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxypropylmethacrylamide), poly( $\alpha$ -hydroxy acid), poly(vinyl alcohol), polyphosphazene, polyoxazoline, poly(N-acryloylmorpholine), such as described in U.S. Pat. No. 5,629,384, which is incorporated by reference herein in its entirety, 25 and copolymers, terpolymers, and mixtures thereof. Although the molecular weight of each chain of the polymer backbone can vary, it is typically in the range of from about 100 Da to about 100,000 Da, often from about 6,000 Da to about 80,000 Da.

[0087] The terms "large-scale" and "industrial-scale" are used interchangeably and refer to a reaction cycle that produces at least about 250 mg, preferably at least about 500 mg, and 30 more preferably at least about 1 gram of glycoconjugate at the completion of a single reaction cycle.

[0088] The term, "glycosyl linking group," as used herein refers to a glycosyl residue that is a fragment of a parent saccharide, generally prepared by oxidation of one or more primary or secondary hydroxyl moieties on the parent saccharide. An exemplary glycosyl linking group is set forth in Formula I, below. As shown in Formula I, the glycosyl linking group covalently joins the modifying group (e.g., PEG moiety, therapeutic moiety, biomolecule) to the molecule to which it is attached. In the methods of the invention, the "glycosyl linking group" is formed by the covalent modification, via an enzymatic glycosylation reaction linking the agent to an amino acid and/or glycosyl residue on the peptide. The glycosyl linking group can be a saccharide-derived structure that is degraded or degraded and modified prior to the addition of the modifying group (e.g., oxidation→Schiff base formation→reduction). Alternatively, a portion of the glycosyl linking group may be intact. For example, when the glycosyl linking group is Gal-SA\* (SA\* is the saccharyl fragment), with Gal attached to a peptide or lipid, the Gal can be intact. The glycosyl linking groups of the invention may be derived from a saccharide by addition of glycosyl unit(s) or removal of one or more glycosyl unit from a parent saccharide structure, followed by coupling a saccharyl fragment of the invention to the newly placed or exposed glycosyl residue.

[0089] The term "targeting moiety," as used herein, refers to species that selectively localize in a particular tissue or region of the body. The localization is mediated by specific recognition of molecular determinants, molecular size of the targeting agent or conjugate, ionic interactions, hydrophobic interactions and the like. Other mechanisms of targeting an agent to a particular tissue or region are known to those of skill in the art. Exemplary targeting moieties include antibodies, antibody fragments, transferrin, HS-glycoprotein, coagulation factors, serum proteins, β-glycoprotein, G-CSF, GM-CSF, M-CSF, EPO and the like.

[0090] As used herein, "therapeutic moiety" means any agent useful for therapy including, but not limited to, antibiotics, anti-inflammatory agents, anti-tumor drugs, cytotoxins, and radioactive agents. "Therapeutic moiety" includes prodrugs of bioactive agents, constructs in which more than one therapeutic moiety is bound to a carrier, e.g., multivalent agents. Therapeutic moiety also includes proteins and constructs that include proteins. Exemplary proteins include, but are not limited to, Erythropoietin (EPO), Granulocyte Colony Stimulating Factor (GCSF), Granulocyte Macrophage Colony Stimulating Factor (GMCSF), Interferon (e.g., Interferon-α, -β, -γ), Interleukin (e.g., Interleukin II), serum proteins (e.g.,

Factors VII, VIIa, VIII, IX, and X), Human Chorionic Gonadotropin (HCG), Follicle Stimulating Hormone (FSH) and Lutenizing Hormone (LH) and antibody fusion proteins (e.g. Tumor Necrosis Factor Receptor ((TNFR)/Fc domain fusion protein)).

[0091] As used herein, "anti-tumor drug" means any agent useful to combat cancer

5 including, but not limited to, cytotoxins and agents such as antimetabolites, alkylating agents, anthracyclines, antibiotics, antimitotic agents, procarbazine, hydroxyurea, asparaginase, corticosteroids, interferons and radioactive agents. Also encompassed within the scope of the term "anti-tumor drug," are conjugates of peptides with anti-tumor activity, e.g. TNF- $\alpha$ . Conjugates include, but are not limited to those formed between a therapeutic protein and a  
10 glycoprotein of the invention. A representative conjugate is that formed between PSGL-1 and TNF- $\alpha$ .

[0092] As used herein, "a cytotoxin or cytotoxic agent" means any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicine, doxorubicin,  
15 daunorubicin, dihydroxy anthracenedione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Other toxins include, for example, ricin, CC-1065 and analogues, the duocarmycins. Still other toxins include diphtheria toxin, and snake venom (e.g., cobra venom).

20 [0093] As used herein, "a radioactive agent" includes any radioisotope that is effective in diagnosing or destroying a tumor. Examples include, but are not limited to, indium-111, cobalt-60. Additionally, naturally occurring radioactive elements such as uranium, radium, and thorium, which typically represent mixtures of radioisotopes, are suitable examples of a radioactive agent. The metal ions are typically chelated with an organic chelating moiety.

25 [0094] Many useful chelating groups, crown ethers, cryptands and the like are known in the art and can be incorporated into the compounds of the invention (e.g., EDTA, DTPA, DOTA, NTA, HDTA, etc. and their phosphonate analogs such as DTPP, EDTP, HDTA, NTP, etc.). See, for example, Pitt *et al.*, "The Design of Chelating Agents for the Treatment of Iron Overload," In, INORGANIC CHEMISTRY IN BIOLOGY AND MEDICINE; Martell, Ed.; American  
30 Chemical Society, Washington, D.C., 1980, pp. 279-312; Lindoy, THE CHEMISTRY OF MACROCYCLIC LIGAND COMPLEXES; Cambridge University Press, Cambridge, 1989; Dugas,

BIOORGANIC CHEMISTRY; Springer-Verlag, New York, 1989, and references contained therein.

[0095] Additionally, a manifold of routes allowing the attachment of chelating agents, crown ethers and cyclodextrins to other molecules is available to those of skill in the art. *See,*

5 for example, Meares *et al.*, "Properties of In Vivo Chelate-Tagged Proteins and Polypeptides." In, MODIFICATION OF PROTEINS: FOOD, NUTRITIONAL, AND PHARMACOLOGICAL ASPECTS;" Feeney, *et al.*, Eds., American Chemical Society, Washington, D.C., 1982, pp. 370-387; Kasina *et al.*, *Bioconjugate Chem.*, **9**: 108-117 (1998); Song *et al.*, *Bioconjugate Chem.*, **8**: 249-255 (1997).

10 [0096] As used herein, "pharmaceutically acceptable carrier" includes any material, which when combined with the conjugate retains the conjugates' activity and is non-reactive with the subject's immune systems. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include  
15 sterile solutions, tablets including coated tablets and capsules. Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known  
20 conventional methods.

[0097] As used herein, "administering" means oral administration, administration as a suppository, topical contact, intravenous, intraperitoneal, intramuscular, intralesional, or subcutaneous administration, administration by inhalation, or the implantation of a slow-release device, *e.g.*, a mini-osmotic pump, to the subject. Adminsitration is by any route  
25 including parenteral and transmucosal (*e.g.*, oral, nasal, vaginal, rectal, or transdermal), particularly by inhalation. Parenteral administration includes, *e.g.*, intravenous, intramuscular, intra-arteriole, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial. Moreover, where injection is to treat a tumor, *e.g.*, induce apoptosis, administration may be directly to the tumor and/or into tissues surrounding the tumor. Other  
30 modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, etc.

[0098] The term “isolated” refers to a material that is substantially or essentially free from components, which are used to produce the material. For conjugates of the invention, the term “isolated” refers to material that is substantially or essentially free from components, which normally accompany the material in the mixture used to prepare the conjugate.

5 “Isolated” and “pure” are used interchangeably. Typically, isolated conjugates of the invention have a level of purity preferably expressed as a range. The lower end of the range of purity for the conjugates is about 60%, about 70% or about 80% and the upper end of the range of purity is about 70%, about 80%, about 90% or more than about 90%.

10 [0099] When the conjugates are more than about 90% pure, their purities are also preferably expressed as a range. The lower end of the range of purity is about 90%, about 92%, about 94%, about 96% or about 98%. The upper end of the range of purity is about 92%, about 94%, about 96%, about 98% or about 100% purity.

[0100] Purity is determined by any art-recognized method of analysis (*e.g.*, band intensity on a silver stained gel, polyacrylamide gel electrophoresis, HPLC, or a similar means).

15 [0101] “Essentially each member of the population,” as used herein, describes a characteristic of a population of peptide conjugates of the invention in which a selected percentage of the modified saccharyl fragments added to a peptide are added to multiple, identical acceptor sites on the peptide. “Essentially each member of the population” speaks to the “homogeneity” of the sites on the peptide conjugated to a modified saccharyl fragment 20 and refers to conjugates of the invention, which are at least about 80%, preferably at least about 90% and more preferably at least about 95% homogenous.

[0102] “Homogeneity,” refers to the structural consistency across a population of acceptor moieties to which the modified saccharyl fragments are conjugated. Thus, in a peptide conjugate of the invention in which each modified saccharyl fragment moiety is conjugated 25 to a site having the same structure as the site to which every other modified saccharyl fragment is conjugated, the peptide conjugate is said to be about 100% homogeneous. Homogeneity is typically expressed as a range. The lower end of the range of homogeneity for the peptide conjugates is about 60%, about 70% or about 80% and the upper end of the range of purity is about 70%, about 80%, about 90% or more than about 90%.

30 [0103] When the peptide conjugates are more than or equal to about 90% homogeneous, their homogeneity is also preferably expressed as a range. The lower end of the range of

homogeneity is about 90%, about 92%, about 94%, about 96% or about 98%. The upper end of the range of purity is about 92%, about 94%, about 96%, about 98% or about 100% homogeneity. The purity of the peptide conjugates is typically determined by one or more methods known to those of skill in the art, e.g., liquid chromatography-mass spectrometry (LC-MS), matrix assisted laser desorption mass time of flight spectrometry (MALDITOF), capillary electrophoresis, and the like.

5 [0104] “Substantially uniform conjugate” or a “substantially uniform conjugation pattern,” when referring to a glycoconjugate species, refers to the percentage of peptide glycosylation sites that are functionalized directly, or through a glycosyl linker, with a modified saccharyl fragment. A substantially uniform conjugation pattern exists if substantially all (as defined below) members of a glycosylation site population intended to bear the modified saccharyl 10 fragment are directly or indirectly functionalized with that fragment.

15 [0105] The term “substantially” in the above definitions of “substantially uniform” generally means at least about 40%, at least about 70%, at least about 80%, or more preferably at least about 90%, and still more preferably at least about 95% of the acceptor moieties for a particular modified saccharyl fragment are modified by that fragment.

[0106] The terms “(glyco)peptide” and “(glyco)lipid,” refer, respectively, to peptide and glycopeptide; and lipid and glycolipid. The terms “peptide” and “lipid” are used generically to refer to both glycosylated and non-glycosylated analogues of these species.

20 **Introduction**

[0107] The present invention provides conjugates bearing one or more modified saccharyl fragment moiety. The modified fragment is attached to an acceptor moiety on a substrate, e.g., an amino acid or glycosyl residue of a peptide or glycopeptide, or onto an aglycone or glycosyl residue of a glycolipid (e.g., sphingosine, ceramide, etc.). Also provided are 25 enzymatically-mediated methods for producing the conjugates of the invention, and activated modified saccharyl fragments of use in the methods. The invention also provides pharmaceutical formulations that include a conjugate formed by a method of the invention.

[0108] Conjugates of the invention are formed between a therapeutic core molecule, e.g., (glyco)peptide, (glyco)lipid, and diverse modifying groups such as water-soluble polymers, 30 therapeutic moieties, diagnostic moieties, targeting moieties and the like. The modifying group is conjugated to the therapeutic species through a saccharyl fragment. Also provided

are conjugates that include two or more peptides linked together through a linker arm, *i.e.*, multifunctional conjugates. The multi-functional conjugates of the invention can include two or more copies of the same peptide or a collection of diverse peptides with different structures and/or properties. In exemplary conjugates according to this embodiment, the 5 linker between the two peptides includes at least one saccharyl fragment, or modified saccharyl fragment as described herein.

[0109] The conjugates of the invention are prepared by the enzymatic conjugation of an activated modified saccharyl fragment to a therapeutic substrate. When the conjugate of the 10 invention is a glycopeptide conjugate, the modified saccharyl fragment is attached directly to an amino acid of a glycosylation site, or to a glycosyl residue attached either directly or indirectly (*e.g.*, through one or more glycosyl residue) to a glycosylation site.

[0110] The invention also provides lipid conjugates in which the modified saccharyl fragment is attached to an aglycone moiety of a lipid or to a glycosyl residue of a glycolipid.

[0111] The modified saccharyl fragment, when interposed between the peptide (or glycosyl 15 residue) and the modifying group, becomes what is referred to herein as a “glycosyl linking group.” Using the exquisite selectivity of enzymes, such as glycosyl transferases, amidases, endoglycanases, endoglycoceramidases, and the like, the present method provides peptides and lipids that bear a desired group at one or more specific locations. Thus, in exemplary conjugates according to the present invention, a modified saccharyl fragment is attached 20 directly to a selected locus on the peptide chain or, alternatively, the modified saccharyl fragment is appended onto a carbohydrate moiety of a glycopeptide. Peptides in which modified saccharyl fragments are bound to both a glycopeptide carbohydrate and directly to an amino acid residue of the peptide backbone are also within the scope of the present invention.

[0112] The methods of the invention make it possible to assemble modified glycopeptides 25 and glycolipids that have a substantially homogeneous derivatization pattern; the enzymes used in the invention are generally selective for a particular glycosyl residue or for particular substituents, or substituent patterns, on a glycosyl residue. The methods are also practical for large-scale production of modified glycopeptide and glycolipid conjugates. In one 30 embodiment the methods of the invention provide a practical means for large-scale preparation of glycopeptide and glycolipid conjugates having preselected uniform derivatization patterns. The methods are particularly well suited for modification of

therapeutic peptides, including but not limited to, glycopeptides that are incompletely glycosylated during production in cell culture cells (*e.g.*, mammalian cells, insect cells, plant cells, fungal cells, yeast cells, or prokaryotic cells) or transgenic plants or animals.

[0113] The methods of the invention also provide conjugates of glycosylated and

5 unglycosylated peptides, and glycolipids, with increased therapeutic half-life due to, for example, reduced clearance rate, or reduced rate of uptake by the immune or reticuloendothelial system (RES). Moreover, the methods of the invention provide a means for masking antigenic determinants on peptides, thus reducing or eliminating a host immune response against the peptide. Selective attachment of targeting agents to a peptide or  
10 glycolipid using an appropriate modified saccharyl fragment can also be used to target the peptide or glycolipid to a particular tissue or cell surface receptor that is specific for the particular targeting agent. Moreover, there is provided a class of peptides and glycolipids that are specifically modified with a therapeutic moiety conjugated through a glycosyl linking group.

15 **The Embodiments**

**Compositions: Glyco-conjugates**

[0114] The present invention provides glyco-conjugates that include a saccharyl fragment functionalized with a modifying group. When the saccharyl fragment is formed by oxidation of a saccharide, *e.g.*, sialic acid, the reagent used to conjugate the modifying group to the

20 oxidized saccharide fragment generally includes a group that reacts with a carbonyl moiety formed during the oxidation.

***Modified saccharyl fragments***

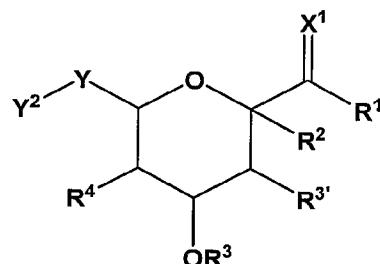
[0115] The present invention provides compounds and methods that are based upon the discovery that enzymes capable of transferring an intact glycosyl moiety to an acceptor substrate are also capable of transferring a modified saccharyl fragment to the acceptor. Accordingly, the invention is not limited by the structure or methods of obtaining appropriate saccharyl fragments or modified saccharyl fragments.

[0116] In an exemplary embodiment, the saccharide fragment is prepared by the oxidative degradation of the parent saccharide. Methods of selectively oxidizing saccharide groups are well known in the art. For example, the periodate ion is of use to cleave vicinal diols, forming the corresponding dialdehyde. Controlled periodate oxidation of the side chain of

sialic acid leads to the formation of an oxidized or truncated side chain bearing an aldehyde. By choosing appropriate conditions, a side chain containing from one to three carbon atoms is produced. See, for example, Chai et al., *Carbohydr. Res.* **239**: 107-115 (1993); and Murray et al., *Carbohydr. Res.* **186**: 107-115 (1989).

5 [0117] The carbonyl moiety introduced into the saccharyl fragment undergoes those reactions generally used for the modification of a carbonyl moiety. For example, modifying groups that include amines are of use as are those that form imines, e.g., hydrazines, semicarbazines and the like. Other typical reactions include the reaction of the carbonyl moiety with ylides (e.g., sulfur and phosphorus), and with Grignard and lithium reagents.

10 [0118] An exemplary modified saccharyl fragment of the invention is formed by the oxidative degradation of the side chain of sialic acid. The oxidation leads to the formation of a carbonyl moiety that is reductively aminated with an amine derivative of a modifying group of interest. Thus, in this embodiment, the invention provides a modified saccharyl fragment having a structure according to Formula I:



15

(I).

[0119] In Formula I, the symbol X<sup>1</sup> represents O or NR<sup>8</sup>. R<sup>8</sup> is a member selected from H, OH, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. Appropriate R<sup>1</sup> groups are selected from OR<sup>9</sup>, NR<sup>9</sup>R<sup>10</sup>, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. The symbols R<sup>9</sup> and R<sup>10</sup> independently represent H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, and C(O)R<sup>11</sup>. R<sup>11</sup> is a group such as substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl.

20 [0120] The symbol R<sup>2</sup> is a member selected from an amino acid residue of a peptide, a carbohydrate moiety attached to an amino acid residue of a peptide, or a carbohydrate moiety attached to an amino acid residue of a peptide through a linker. Exemplary linkers include one or more additional carbohydrate moieties in addition to that of R<sup>2</sup>. R<sup>3</sup> is a member

selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. R<sup>3'</sup> is a member selected from H, OR<sup>4'</sup>, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. R<sup>4</sup> and R<sup>4'</sup> are members independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, OH and

5 NHC(O)R<sup>12</sup>. R<sup>12</sup> is selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl and NR<sup>13</sup>R<sup>14</sup>, in which R<sup>13</sup> and R<sup>14</sup> are members independently selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. In an exemplary embodiment, R<sup>3'</sup> is H.

10 [0121] Y is the residue of the sialic acid side chain remaining following oxidation and further chemical modification. Exemplary groups for Y include CH<sub>2</sub>, CH(OH)CH<sub>2</sub>, CH(OH)CH(OH)CH<sub>2</sub> when the oxidation leads to formation of an aldehyde that is subsequently reductively aminated. When the aldehyde is converted to an imine species, or when the product results from addition of a phosphorus or sulfur ylide, Y is typically CH, 15 CH(OH)CH or CH(OH)CH(OH)CH. When the aldehyde is reacted with a Grignard or lithium reagent, exemplary Y groups include CH(OH), CH(OH)CH(OH), CH(OH)CH(OH)CH(OH) or an elimination product thereof, e.g., dehydration product.

[0122] The symbol Y<sup>2</sup> represents groups formed by addition to the carbonyl moiety of the fragment. Y<sup>2</sup> includes at least one modifying group e.g., biomolecule, therapeutic moiety, 20 diagnostic moiety, and a polymeric modifying group, as exemplified by the term R<sup>6a</sup>. Exemplary identities for Y<sup>2</sup> include substituted alkyl (e.g., formed by Wittig, Grignard or other appropriate chemistries), R<sup>6</sup> and nitrogen-containing species, e.g., NR<sup>6</sup>R<sup>7</sup> or R<sup>6</sup>R<sup>7</sup>N-N=. R<sup>6</sup> and R<sup>7</sup> are independently H, C(O)R<sup>6b</sup> or -L<sup>a</sup>-R<sup>6b</sup> wherein R<sup>6b</sup> is H or R<sup>6a</sup> and L<sup>a</sup> is selected 25 from a bond and a linker group. In an exemplary embodiment, Y<sup>2</sup> is N(R<sup>6</sup>)-L<sup>a</sup>-(m-PEG)<sub>s</sub> wherein L<sup>a</sup> is a linker moiety which is a member selected from an amino acid residue and a peptidyl residue; and the index s is an integer from 1 to 3.

[0123] When Y<sup>2</sup> is substituted or unsubstituted alkyl, e.g., an alkene species formed by a Wittig reaction, or saturated species formed by Grignard or lithium chemistries, Y<sup>2</sup> includes at least one modifying group (water-soluble or -insoluble polymer) as exemplified by the 30 term R<sup>6a</sup>.

[0124] In an exemplary embodiment, the modified saccharyl fragment is prepared by reacting a carbonyl-containing saccharyl fragment with a Wittig reagent that includes within

its structure a water-soluble polymer, e.g., m-PEG. Wittig reagents of m-PEG are readily formed by reaction of chloro-m-PEG with  $\text{PPh}_3$  and treating the resulting adduct with a base to form the ylide. Other ylides of use in forming the compounds of the invention are prepared by deprotonating an alkyl phosphonate according to the Arbuzov reaction and reacting the carbonyl moiety of the saccharyl fragment with this ylide under conditions appropriate for the Horner-Emmons reaction.

5 [0125] Grignard reagents of use in present invention, e.g. m-PEGMgBr, are readily prepared according to art-recognized methods. For example, m-PEG-Br is reacted with Mg under anhydrous conditions.

10 [0126] In another exemplary embodiment, the carbonyl-containing saccharyl fragment is reductively aminated with ammonia. The resulting amine is alkylated or acylated with a selected modifying group, e.g., m-PEG or branched m-PEG.

[0127] Typically, the saccharyl fragment is a monosaccharide; however, because the side chain of sialic acid is selectively oxidized in the presence of the vicinal diols of other

15 saccharides, the present invention is not limited to the use of modified sialic acid, but is of use with sialic acid fragment-containing oligosaccharides and polysaccharides as well.

[0128] In another aspect, the invention provides an activated modified saccharyl fragment that is of use in the methods of the invention. An exemplary activated modified saccharyl fragment includes an activated leaving group. As used herein, the term "activated leaving 20 group" refers to those moieties, which are easily displaced in enzyme-regulated nucleophilic substitution reactions. Many activated sugars are known in the art. See, for example, Vocablo et al., In CARBOHYDRATE CHEMISTRY AND BIOLOGY, Vol. 2, Ernst *et al.* Ed., Wiley-VCH Verlag: Weinheim, Germany, 2000; Kodama *et al.*, *Tetrahedron Lett.* **34**: 6419 (1993); Lougheed, *et al.*, *J. Biol. Chem.* **274**: 37717 (1999)).

25 [0129] In an exemplary embodiment, according to this aspect, the saccharyl fragment has a structure according to Formula I in which  $R^2$  is an activating group. An exemplary activating group is a nucleotide, forming a nucleotide sugar in which the sugar moiety is the saccharyl fragment.  $R^2$  can also be a leaving group (activating group), such as a halogen, sulfonate ester and the like.

30 [0130] An exemplary activated leaving group is a nucleotide, which can be utilized to add the modified saccharyl fragment to an acceptor moiety on the substrate. Exemplary sugar

nucleotides present in the compounds of the invention include nucleotide mono-, di- or triphosphates or analogs thereof. In a preferred embodiment, the modified saccharyl fragment nucleotide is selected from a UDP-glycoside, CMP-glycoside, or a GDP-glycoside. Even more preferably, the modified saccharyl fragment nucleotide is selected from analogues of UDP-galactose, UDP-galactosamine, UDP-glucose, UDP-glucosamine, GDP-mannose, GDP-fucose, CMP-sialic acid or CMP-NeuAc in which the saccharyl moiety (other than the nucleotide ribose) is a saccharyl fragment bearing a modifying group.

[0131] In an exemplary embodiment, one or more sugar nucleotides or modified sugar nucleotides are used in conjunction with a glycosyltransferase.

[0132] In other embodiments, the activating moiety is an activated leaving group other than a nucleotide. Examples of non-nucleotide activating groups include fluoro, chloro, bromo, tosylate ester, mesylate ester, triflate ester and the like. Preferred activated leaving groups, for use in the present invention, are those that do not significantly sterically encumber the enzymatic transfer of the glycoside to the acceptor. Accordingly, preferred embodiments of activated glycoside derivatives include glycosyl fluorides and glycosyl mesylates, with glycosyl fluorides being particularly preferred. Among the glycosyl fluorides,  $\alpha$ -galactosyl fluoride,  $\alpha$ -mannosyl fluoride,  $\alpha$ -glucosyl fluoride,  $\alpha$ -fucosyl fluoride,  $\alpha$ -xylosyl fluoride,  $\alpha$ -sialyl fluoride,  $\alpha$ -N-acetylglucosaminyl fluoride,  $\alpha$ -N-acetylgalactosaminyl fluoride,  $\beta$ -galactosyl fluoride,  $\beta$ -mannosyl fluoride,  $\beta$ -glucosyl fluoride,  $\beta$ -fucosyl fluoride,  $\beta$ -xylosyl fluoride,  $\beta$ -sialyl fluoride,  $\beta$ -N-acetylglucosaminyl fluoride and  $\beta$ -N-acetylgalactosaminyl fluoride are most preferred.

[0133] By way of illustration, glycosyl fluorides can be prepared from the saccharyl fragment or modified saccharyl fragment by first acetylating the sugar and then treating it with HF/pyridine. This generates the thermodynamically most stable anomer of the protected (acetylated) glycosyl fluoride (*i.e.*, the  $\alpha$ -glycosyl fluoride). If the less stable anomer (*i.e.*, the  $\beta$ -glycosyl fluoride) is desired, it can be prepared by converting the peracetylated sugar with HBr/HOAc or with HCl to generate the anomeric bromide or chloride. This intermediate is reacted with a fluoride salt such as silver fluoride to generate the glycosyl fluoride. Acetylated glycosyl fluorides may be deprotected by reaction with mild (catalytic) base in methanol (*e.g.* NaOMe/MeOH). In addition, many glycosyl fluorides are commercially available.

**[0134]** Other activated glycosyl derivatives can be prepared using conventional methods known to those of skill in the art. For example, glycosyl mesylates can be prepared by treatment of the fully benzylated hemiacetal form of the sugar with mesyl chloride, followed by catalytic hydrogenation to remove the benzyl groups.

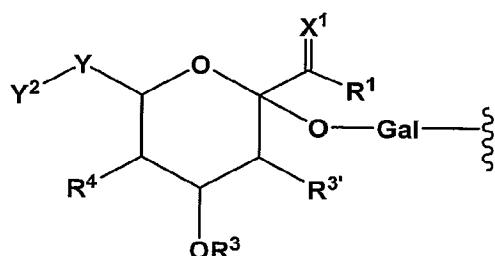
5   **[0135]** In an exemplary embodiment, one or more activated glycosyl derivative such as those set forth above is used in conjunction with an enzyme that is a mutant of a degradative enzyme; mutated to enhance its activity forming glycosidic and amino-glycosidic bonds relative to the activity of the wild-type, which predominantly cleave these bonds. Enzymes of use in this embodiment include those described in WO03/046150, WO03/045980, and  
10   their US counterpart patent applications).

15   **[0136]** In addition to including a moiety according to Formula I, the conjugates of the invention can include one or more additional modified saccharyl fragment appended to an amino acid, aglycone or glycosyl residue of the conjugate. The structure and preparation of exemplary modified saccharyl fragments that are of use in combination with the modified saccharyl fragment of the invention are also disclosed in WO03/031464 and related U.S. and PCT applications.

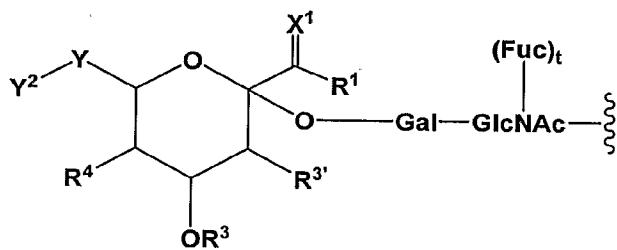
### Sugars

20   **[0137]** Any sugar can be utilized as the sugar core of the modified saccharyl fragment conjugates of the invention. Exemplary sugar cores that are useful in forming the compositions of the invention include, but are not limited to, sialic acid, glucose, galactose, and mannose and N-acetyl analogues of these sugars. Also of use are fucose, xylose, ribose, and arabinose. Also encompassed within the invention are species in which the sugar core is a disaccharide, an oligosaccharide or a polysaccharide.

25   **[0138]** The invention provides a peptide or lipid conjugate that includes a glycosyl linking group having the formula:

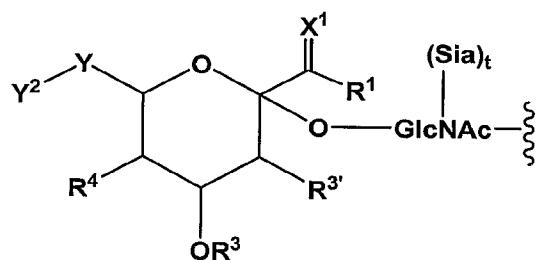


**[0139]** In other embodiments, the glycosyl linking group has the formula:



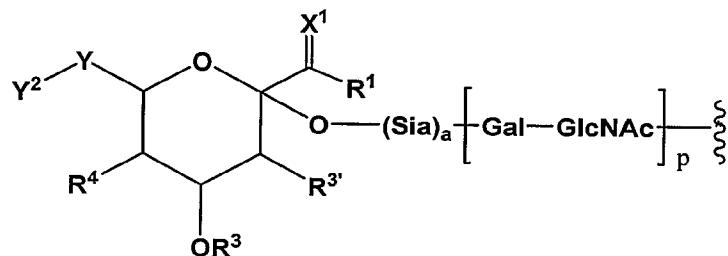
in which the index t is 0 or 1.

**[0140]** In a still further exemplary embodiment, the glycosyl linking group has the  
5 formula:



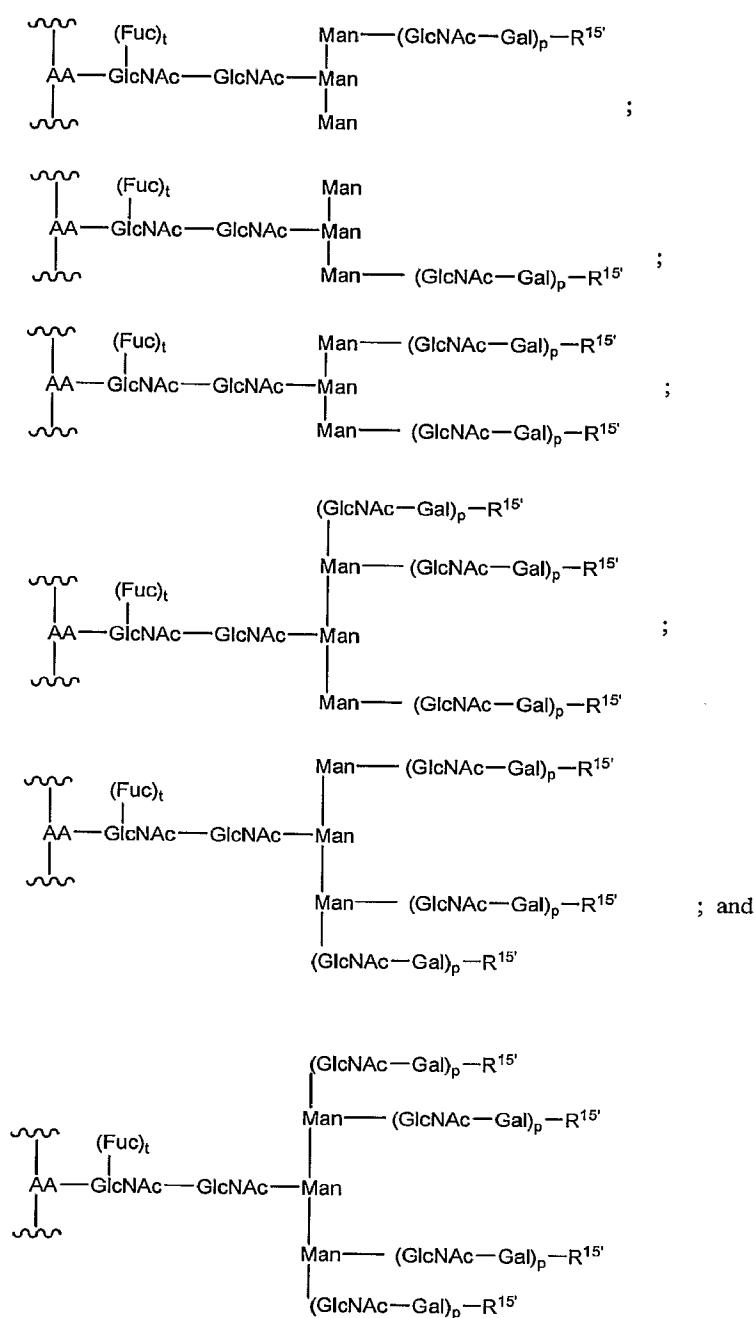
in which the index t is 0 or 1.

**[0141]** In yet another embodiment, the glycosyl linking group has the formula:



10 in which the index p represents an integer from 1 to 10; and a is either 0 or 1.

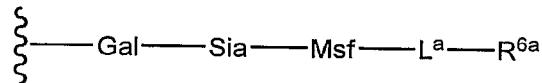
**[0142]** In an exemplary embodiment, the invention provides a glycoPEGylated peptide conjugate which is selected from the formulae set forth below:



[0143] In the formulae above, the index t is an integer from 0 to 1 and the index p is an integer from 1 to 10. The symbol R<sup>15'</sup> represents H, OH (e.g., Gal-OH), a modified saccharyl fragment (Msf), a Msf which comprises -L<sup>a</sup>-R<sup>6a</sup>, a Msf which comprises R<sup>6a</sup>, wherein R<sup>6a</sup> is a polymeric modifying group, or a sialyl moiety to which is bound a modified saccharyl fragment which comprises -L<sup>a</sup>-R<sup>6a</sup> (e.g., Sia-Msf-L<sup>a</sup>-R<sup>6a</sup>), or a sialyl moiety to which is bound a modified saccharyl fragment which comprises R<sup>6a</sup>, (e.g., Sia-Msf-R<sup>6a</sup>) ("Sia-Msf<sup>p</sup>"). Exemplary polymer modified saccharyl moieties have a structure according to Formula I. An

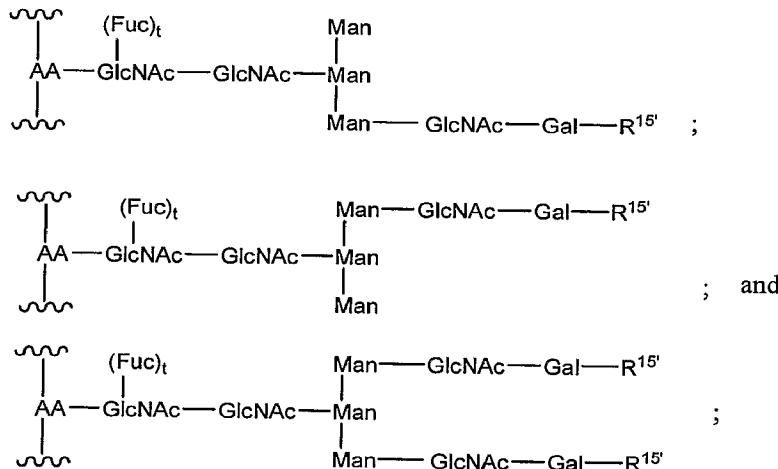
exemplary peptide conjugate of the invention will include at least one glycan having a R<sup>15'</sup> that includes a structure according to Formula I. In a further exemplary embodiment, the oxygen is attached to the carbon at position 3 of a galactose residue. In an exemplary embodiment, the modified sialic acid is linked α2,3-to the galactose residue. In another 5 exemplary embodiment, the sialic acid is linked α2,6-to the galactose residue.

**[0144]** In an exemplary embodiment, R<sup>15'</sup> is a sialyl moiety to which is bound a modified saccharyl fragment which comprises -L<sup>a</sup>-R<sup>6a</sup>, or R<sup>6a</sup>, (e.g., Sia-Msf-L<sup>a</sup>-R<sup>6a</sup>) ("Sia-Msf<sup>p</sup>"). Here, the glycosyl linking group is linked to a galactosyl moiety through a sialyl moiety:



10 An exemplary species according to this motif is prepared by conjugating Msf-L<sup>a</sup>-R<sup>6a</sup> to a terminal sialic acid of a glycan using an enzyme that forms Sia-Sia bonds, e.g., CST-II, ST8Sia-II, ST8Sia-III and ST8Sia-IV.

**[0145]** In another exemplary embodiment, the glycans on the peptide conjugates have a formula that is selected from the group:



15

and combinations thereof.

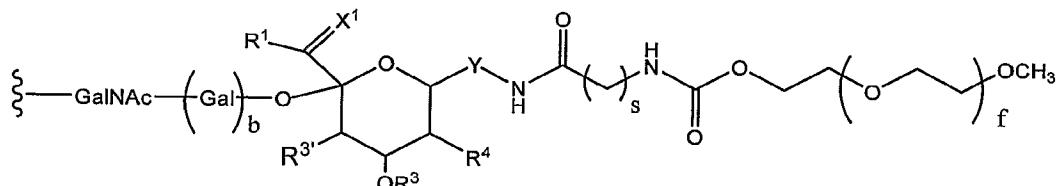
**[0146]** In each of the formulae above, R<sup>15'</sup> is as discussed above. Moreover, an exemplary peptide conjugate of the invention will include at least one glycan with an R<sup>15'</sup> moiety having a structure according to Formula I.

**[0147]** In another exemplary embodiment, the glycosyl linking group has a formula according to:



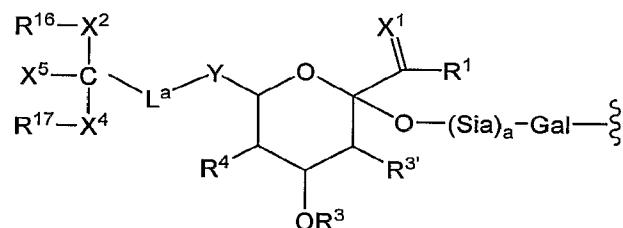
wherein  $R^{15}$  includes a modified saccharyl fragment; and the index p is an integer selected from 1 to 10.

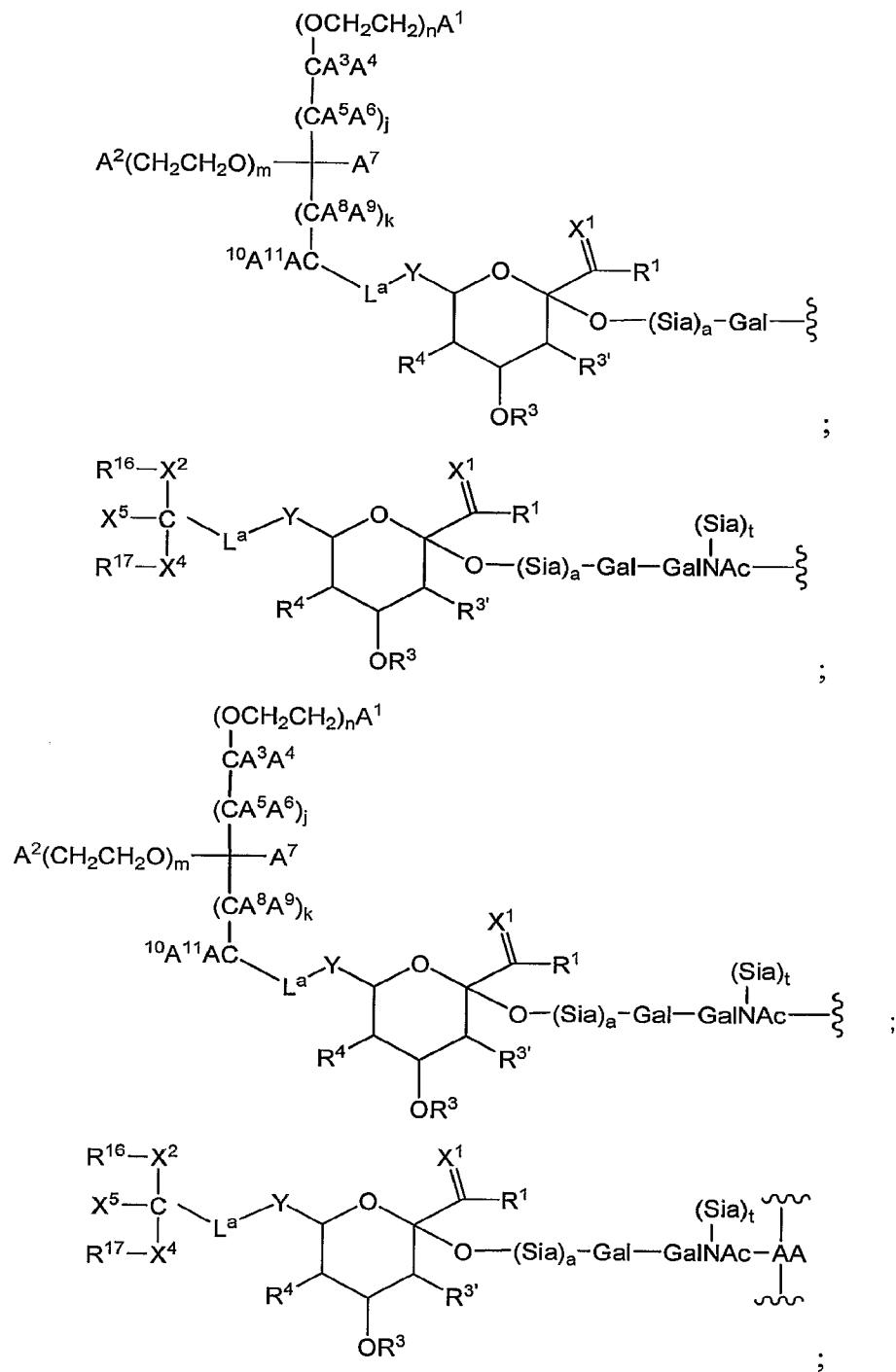
**[0148]** In an exemplary embodiment, the modified saccharyl fragment has the formula:

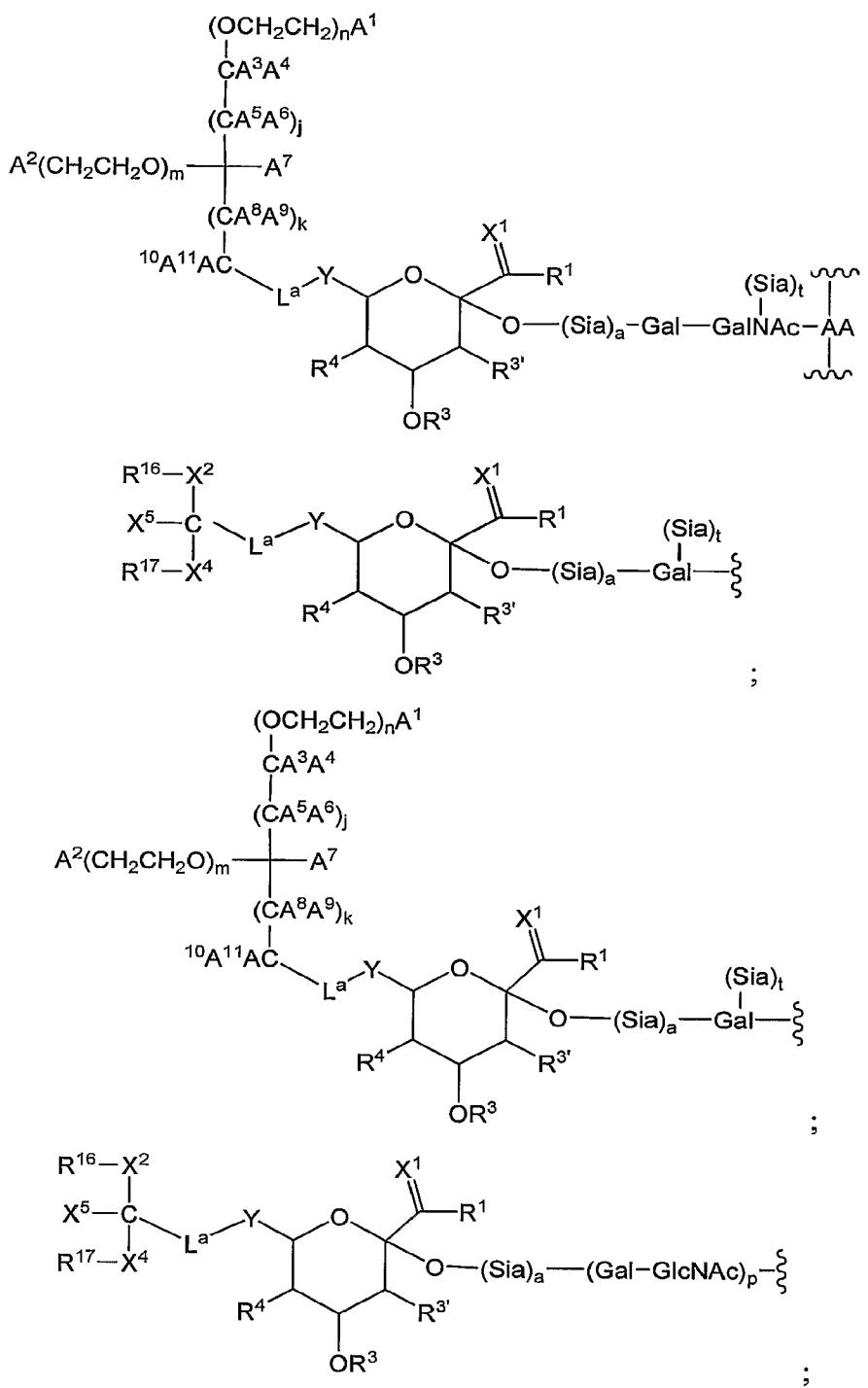


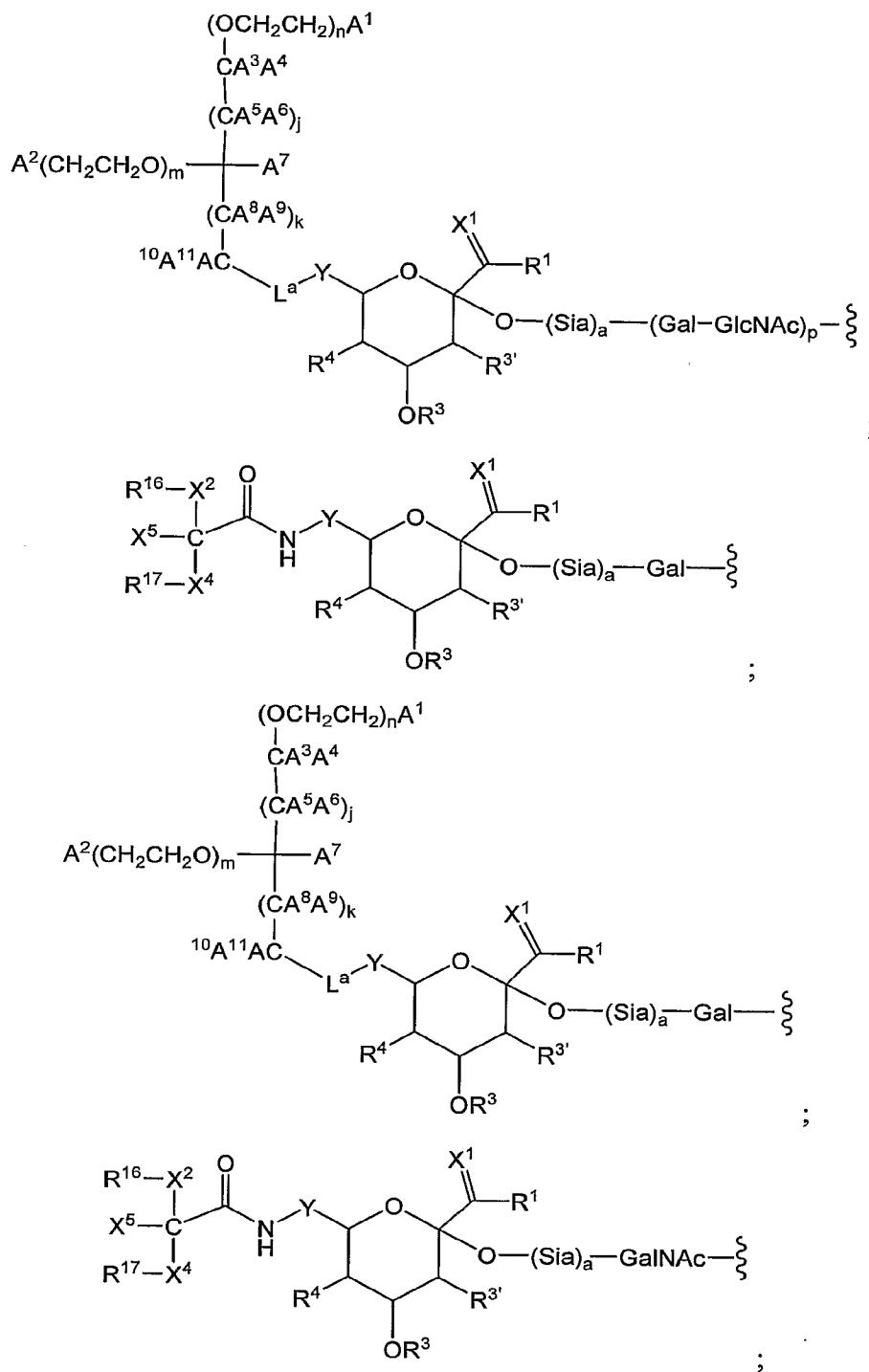
in which b is an integer from 0 to 1. The index s represents an integer from 1 to 10; and the index f represents an integer from 1 to 2500.

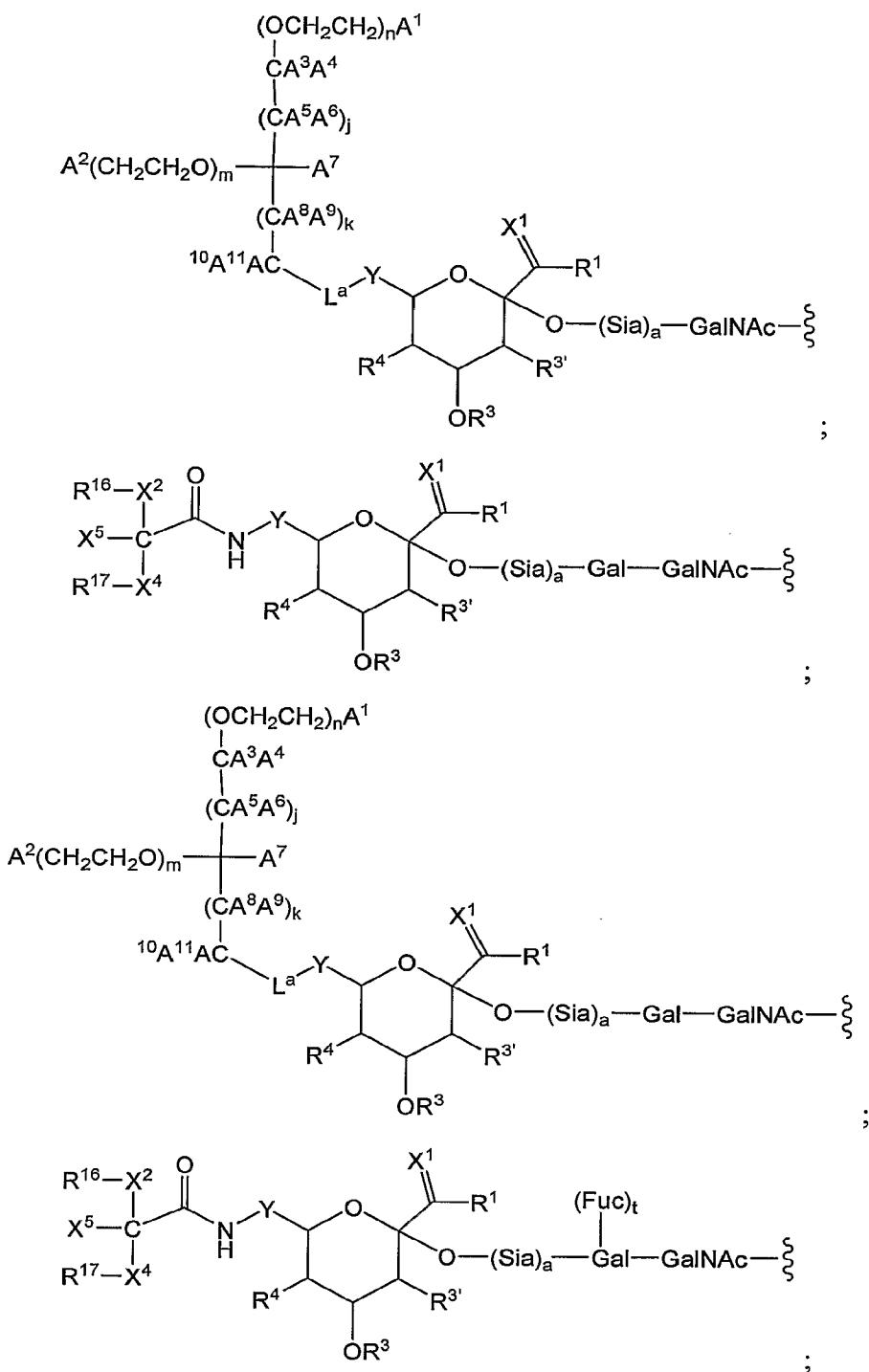
**[0149]** In another exemplary embodiment, the peptide conjugate comprises a glycosyl moiety selected from the formulae:

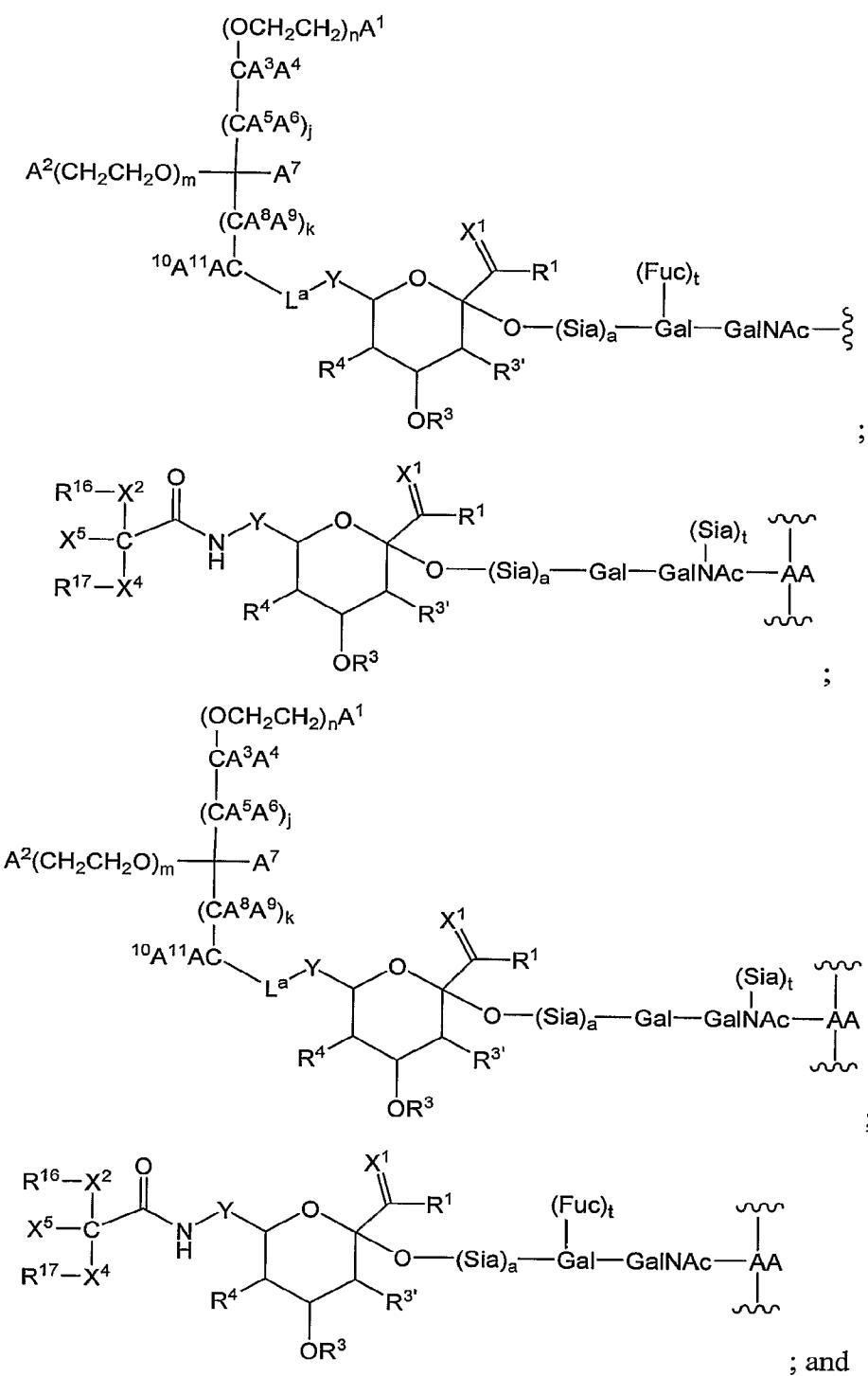


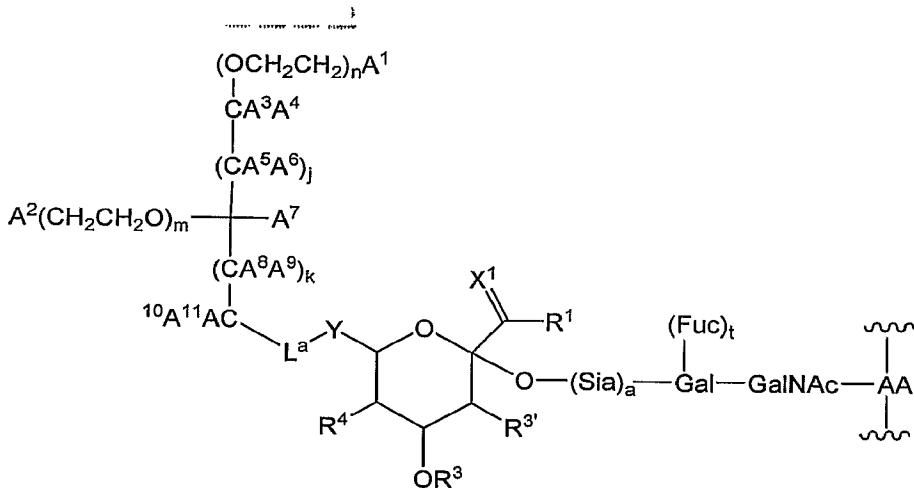












in which the index p is an integer from 1 to 10. The indices t and a are independently selected from 0 or 1. The indices m and n are integers independently selected from 0 to 5000.

The indices j and k are integers independently selected from 0 to 20. A<sup>1</sup>, A<sup>2</sup>, A<sup>3</sup>, A<sup>4</sup>, A<sup>5</sup>, A<sup>6</sup>,

5 A<sup>7</sup>, A<sup>8</sup>, A<sup>9</sup>, A<sup>10</sup> and A<sup>11</sup> are members independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, -NA<sup>12</sup>A<sup>13</sup>, -OA<sup>12</sup> and -SiA<sup>12</sup>A<sup>13</sup>. A<sup>12</sup> and A<sup>13</sup> are members independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl. AA is an amino acid residue of the peptide. Each of these groups can be included as components of the mono-, bi-, tri- and tetra-antennary saccharide structures set forth above. L<sup>a</sup> is a linker that results from the reaction of the polymer modifying group moiety

10 and the modified saccharyl fragment. Exemplary linking groups include substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl moieties. An exemplary component of the linker is an acyl moiety. Another exemplary linking group is an amino acid (e.g., cysteine, serine, lysine, and short oligopeptides, e.g., Lys-Lys, Lys-Lys-Lys, Cys-Lys, Ser-Lys, etc.).

15

## 20 Modifying Groups

**[0150]** The peptide conjugates of the invention comprise a modifying group. This group can be covalently attached to a peptide through an amino acid or a glycosyl linking group. “Modifying groups” can encompass a variety of structures including targeting moieties, therapeutic moieties, biomolecules. Additionally, “modifying groups” include polymeric

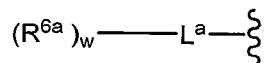
modifying groups, which can alter a property of the peptide such as its bioavailability or its half-life in the body.

[0151] In an exemplary embodiment, the modifying group is a targeting agent that localizes selectively in a particular tissue due to the presence of a targeting agent as a component of the conjugate. In an exemplary embodiment, the targeting agent is a protein. Exemplary proteins include transferrin (brain, blood pool), HS-glycoprotein (bone, brain, blood pool), antibodies (brain, tissue with antibody-specific antigen, blood pool), coagulation factors V-XII (damaged tissue, clots, cancer, blood pool), serum proteins, *e.g.*,  $\alpha$ -acid glycoprotein, fetuin,  $\alpha$ -fetal protein (brain, blood pool),  $\beta$ 2-glycoprotein (liver, atherosclerosis plaques, brain, blood pool), G-CSF, GM-CSF, M-CSF, and EPO (immune stimulation, cancers, blood pool, red blood cell overproduction, neuroprotection), albumin (increase in half-life), and lipoprotein E.

[0152] For the purposes of convenience, the modifying groups in the remainder of this section will be largely based on polymeric modifying groups such as water soluble and water insoluble polymers. However, one of skill in the art will recognize that other modifying groups, such as targeting moieties, therapeutic moieties and biomolecules, could be used in place of the polymeric modifying groups.

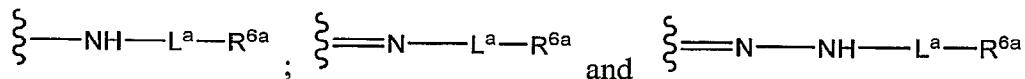
#### Linkers of the Modifying Groups

[0153] The linkers of the modifying group serve to attach the modifying group (*ie* polymeric modifying groups, targeting moieties, therapeutic moieties and biomolecules) to the glycosyl linking group. In an exemplary embodiment, the polymeric modifying group is bound to a glycosyl linking group, generally through a heteroatom, *e.g.*, nitrogen, on the core through a linker,  $L^a$ , as shown below:

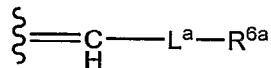


$R^{6a}$  is the polymeric modifying moiety and  $L^a$  is selected from a bond and a linking group. The index  $w$  represents an integer selected from 1-6, preferably 1-3 and more preferably 1-2. Exemplary linking groups include substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl moieties. An exemplary component of the linker is an acyl moiety.

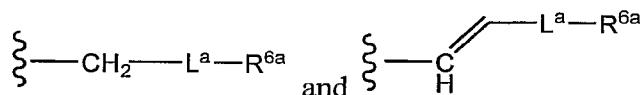
[0154] In an exemplary embodiment, the invention has a structure according to Formula I above, in which  $Y^2$  is selected from the formulae:



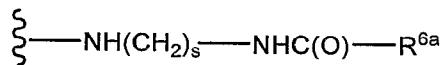
[0155] In another exemplary embodiment, the compound is the product of a Wittig reaction and Y<sup>2</sup> has the formula:



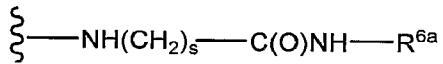
5 [0156] In another exemplary embodiment, the compound is formed from a reaction of the modified glycosyl linking fragment with a Grignard or lithium reagent and Y<sup>2</sup> has a structure selected from the formulae:



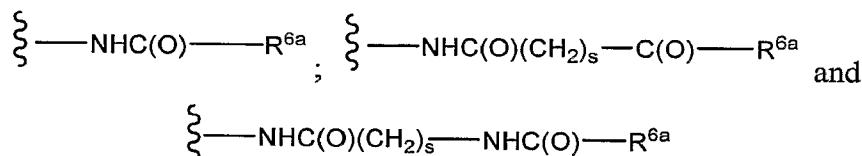
10 [0157] In yet another exemplary embodiment, the glycosyl linking group and the polymeric modifying group are linked through a diamine. In an exemplary compound according to this aspect of the invention Y<sup>2</sup> has the formula:



15 [0158] In another exemplary embodiment the glycosyl linking group and the modifying group are linked through an aminocarboxylic acid. In an exemplary compound according to this aspect of the invention Y<sup>2</sup> has the formula:

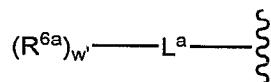


20 [0159] In yet another exemplary embodiment the aldehyde containing glycosyl linking group is reductively aminated with ammonia and the resulting amine is used to attach the polymeric modifying group, thereby forming an amide bond. In this aspect of the invention Y<sup>2</sup> is selected from the formulae:



in which the index s is an integer from 0 to 20.

**[0160]** In an exemplary embodiment, the polymeric modifying group -linker construct is a branched structure that includes two or more polymeric chains attached to central moiety. In this embodiment, the construct has the formula:



5 in which  $R^{6a}$  and  $L^a$  are as discussed above and  $w'$  is an integer from 2 to 6, preferably from 2 to 4 and more preferably from 2 to 3.

**[0161]** When  $L^a$  is a bond it is formed between a reactive functional group on a precursor of  $R^{6a}$  and a reactive functional group of complementary reactivity on the saccharyl core.

10 When  $L^a$  is a non-zero order linker, a precursor of  $L^a$  can be in place on the glycosyl moiety prior to reaction with the  $R^{6a}$  precursor. Alternatively, the precursors of  $R^{6a}$  and  $L^a$  can be incorporated into a preformed cassette that is subsequently attached to the glycosyl moiety. As set forth herein, the selection and preparation of precursors with appropriate reactive functional groups is within the ability of those skilled in the art. Moreover, coupling the precursors proceeds by chemistry that is well understood in the art.

15 **[0162]** In an exemplary embodiment,  $L^a$  is a linking group that is formed from an amino acid, or small peptide (*e.g.*, 1-4 amino acid residues) providing a modified sugar in which the polymeric modifying group is attached through a substituted alkyl linker. Exemplary linkers include glycine, lysine, serine and cysteine. The PEG moiety can be attached to the amine moiety of the linker through an amide or urethane bond. The PEG is linked to the sulfur or 20 oxygen atoms of cysteine and serine through thioether or ether bonds, respectively.

#### Water-Soluble Polymers

**[0163]** Many water-soluble polymers are known to those of skill in the art and are useful in practicing the present invention. The term water-soluble polymer encompasses species such as saccharides (*e.g.*, dextran, amylose, hyalouronic acid, poly(sialic acid), heparans, heparins, etc.); poly (amino acids), *e.g.*, poly(aspartic acid) and poly(glutamic acid); nucleic acids; synthetic polymers (*e.g.*, poly(acrylic acid), poly(ethers), *e.g.*, poly(ethylene glycol); peptides, proteins, and the like. The present invention may be practiced with any water-soluble polymer with the sole limitation that the polymer must include a point at which the remainder of the conjugate can be attached.

[0164] Methods for activation of polymers can also be found in WO 94/17039, U.S. Pat. No. 5,324,844, WO 94/18247, WO 94/04193, U.S. Pat. No. 5,219,564, U.S. Pat. No. 5,122,614, WO 90/13540, U.S. Pat. No. 5,281,698, and more WO 93/15189, and for conjugation between activated polymers and peptides, e.g. Coagulation Factor VIII (WO 94/15625), hemoglobin (WO 94/09027), oxygen carrying molecule (U.S. Pat. No. 4,412,989), ribonuclease and superoxide dismutase (Veronese *et al.*, *App. Biochem. Biotech.* 11: 141-45 (1985)).

[0165] Exemplary water-soluble polymers are those in which a substantial proportion of the polymer molecules in a sample of the polymer are of approximately the same molecular weight; such polymers are "homodisperse."

[0166] The present invention is further illustrated by reference to a poly(ethylene glycol) conjugate. Several reviews and monographs on the functionalization and conjugation of PEG are available. See, for example, Harris, *Macromol. Chem. Phys.* C25: 325-373 (1985); Scouten, *Methods in Enzymology* 135: 30-65 (1987); Wong *et al.*, *Enzyme Microb. Technol.* 14: 866-874 (1992); Delgado *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems* 9: 249-304 (1992); Zalipsky, *Bioconjugate Chem.* 6: 150-165 (1995); and Bhadra, *et al.*, *Pharmazie*, 57:5-29 (2002). Routes for preparing reactive PEG molecules and forming conjugates using the reactive molecules are known in the art. For example, U.S. Patent No. 5,672,662 discloses a water soluble and isolatable conjugate of an active ester of a polymer acid selected from linear or branched poly(alkylene oxides), poly(oxyethylated polyols), poly(olefinic alcohols), and poly(acrylomorpholine).

[0167] U.S. Patent No. 6,376,604 sets forth a method for preparing a water-soluble 1-benzotriazolylcarbonate ester of a water-soluble and non-peptidic polymer by reacting a terminal hydroxyl of the polymer with di(1-benzotriazoyl)carbonate in an organic solvent. The active ester is used to form conjugates with a biologically active agent such as a protein or peptide.

[0168] WO 99/45964 describes a conjugate comprising a biologically active agent and an activated water soluble polymer comprising a polymer backbone having at least one terminus linked to the polymer backbone through a stable linkage, wherein at least one terminus comprises a branching moiety having proximal reactive groups linked to the branching moiety, in which the biologically active agent is linked to at least one of the proximal reactive groups. Other branched poly(ethylene glycols) are described in WO 96/21469, U.S. Patent

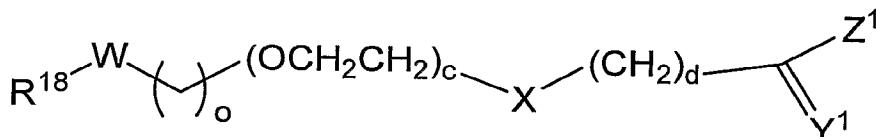
No. 5,932,462 describes a conjugate formed with a branched PEG molecule that includes a branched terminus that includes reactive functional groups. The free reactive groups are available to react with a biologically active species, such as a protein or peptide, forming conjugates between the poly(ethylene glycol) and the biologically active species. U.S. Patent 5 No. 5,446,090 describes a bifunctional PEG linker and its use in forming conjugates having a peptide at each of the PEG linker termini.

**[0169]** Conjugates that include degradable PEG linkages are described in WO 99/34833; and WO 99/14259, as well as in U.S. Patent No. 6,348,558. Such degradable linkages are applicable in the present invention.

10 **[0170]** The art-recognized methods of polymer activation set forth above are of use in the context of the present invention in the formation of the branched polymers set forth herein and also for the conjugation of these branched polymers to other species, *e.g.*, sugars, sugar nucleotides and the like.

15 **[0171]** An exemplary water-soluble polymer is poly(ethylene glycol), *e.g.*, methoxy-poly(ethylene glycol). The poly(ethylene glycol) used in the present invention is not restricted to any particular form or molecular weight range. For unbranched poly(ethylene glycol) molecules the molecular weight is preferably between 500 and 100,000. A molecular weight of 2000-60,000 is preferably used and preferably of from about 5,000 to about 40,000.

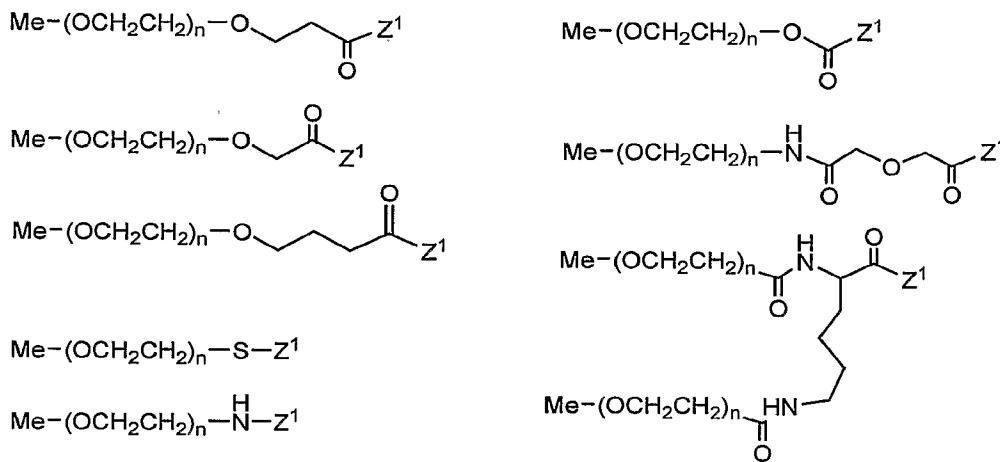
20 **[0172]** In an exemplary embodiment, poly(ethylene glycol) molecules of the invention include, but are not limited to, those species set forth below.



in which R<sup>18</sup> is H, substituted or unsubstituted alkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted heteroalkyl, *e.g.*, acetal, OHC-, H<sub>2</sub>N-CH<sub>2</sub>CH<sub>2</sub>-, HS-CH<sub>2</sub>CH<sub>2</sub>-, and-(CH<sub>2</sub>)<sub>q</sub>C(Y<sup>1</sup>)Z<sup>2</sup>; -sugar-nucleotide, or protein. The index "c" represents an integer from 1 to 2500. The indeces d, o, and q independently represent integers from 0 to 20. The symbol Z<sup>1</sup> represents OH, NH<sub>2</sub>, halogen, S-R<sup>19</sup>, the alcohol portion of activated esters, -(CH<sub>2</sub>)<sub>d1</sub>C(Y<sup>3</sup>)V, -(CH<sub>2</sub>)<sub>d1</sub>U(CH<sub>2</sub>)<sub>g</sub>C(Y<sup>3</sup>)V, sugar-nucleotide, protein, and leaving groups, *e.g.*, imidazole, p-nitrophenyl, HOBT, tetrazole, halide. The symbols X, Y<sup>1</sup>, Y<sup>3</sup>, W, U

independently represent the moieties O, S, N-R<sup>20</sup>. The symbol V represents OH, NH<sub>2</sub>, halogen, S-R<sup>21</sup>, the alcohol component of activated esters, the amine component of activated amides, sugar-nucleotides, and proteins. The indeces d1, g and v are members independently selected from the integers from 0 to 20. The symbols R<sup>19</sup>, R<sup>20</sup> and R<sup>21</sup> independently 5 represent H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heterocycloalkyl and substituted or unsubstituted heteroaryl.

[0173] In other exemplary embodiments, the poly(ethylene glycol) molecule is selected from the following:

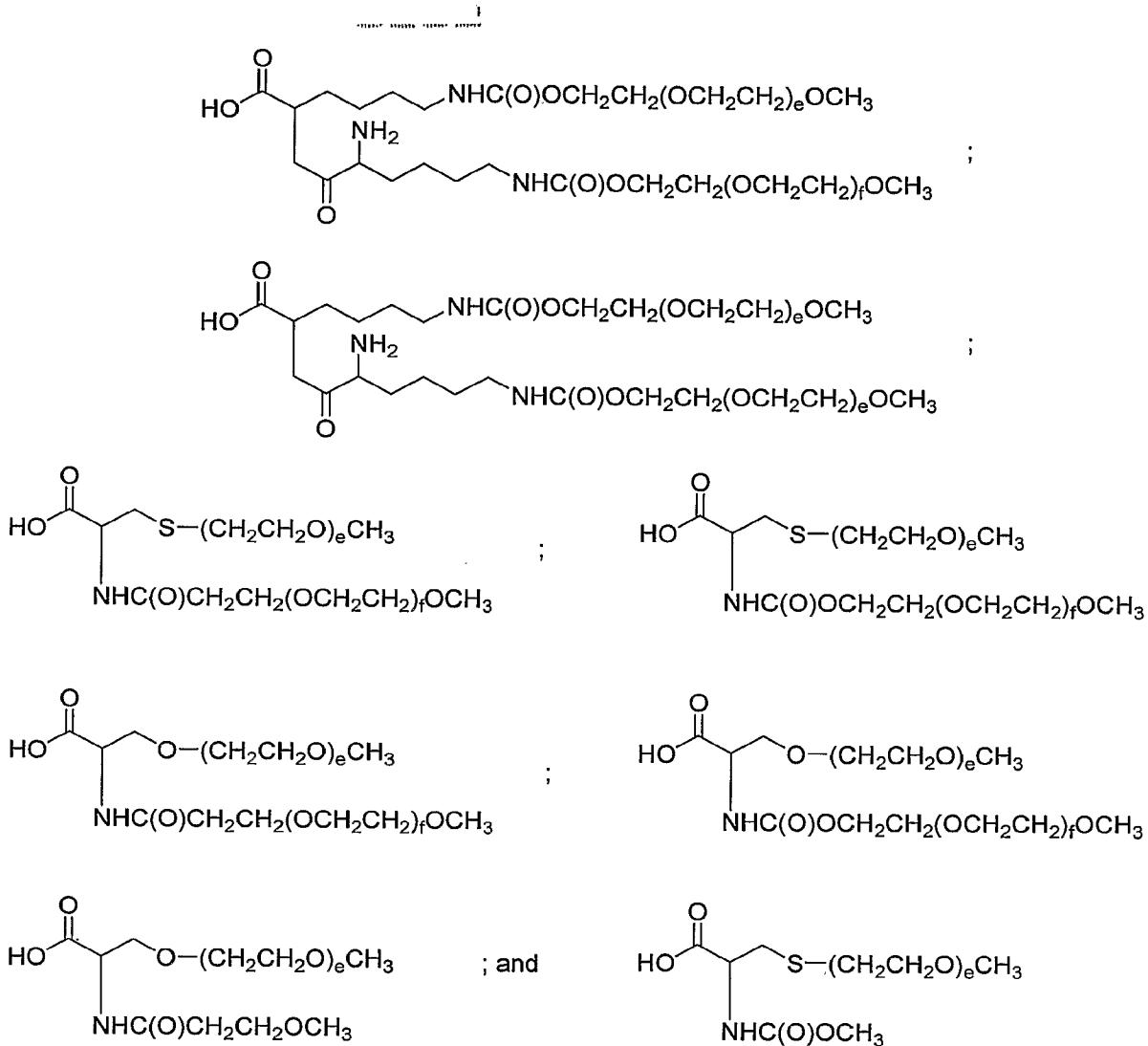


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[0174] In another embodiment the poly(ethylene glycol) is a branched PEG having more than one PEG moiety attached. Examples of branched PEGs are described in U.S. Pat. No. 5,932,462; U.S. Pat. No. 5,342,940; U.S. Pat. No. 5,643,575; U.S. Pat. No. 5,919,455; U.S. Pat. No. 6,113,906; U.S. Pat. No. 5,183,660; WO 02/09766; Kodera Y., *Bioconjugate Chemistry* 5: 283-288 (1994); and Yamasaki et al., *Agric. Biol. Chem.*, 52: 2125-2127, 1998. In a preferred embodiment the molecular weight of each poly(ethylene glycol) of the branched PEG is less than or equal to 40,000 daltons.

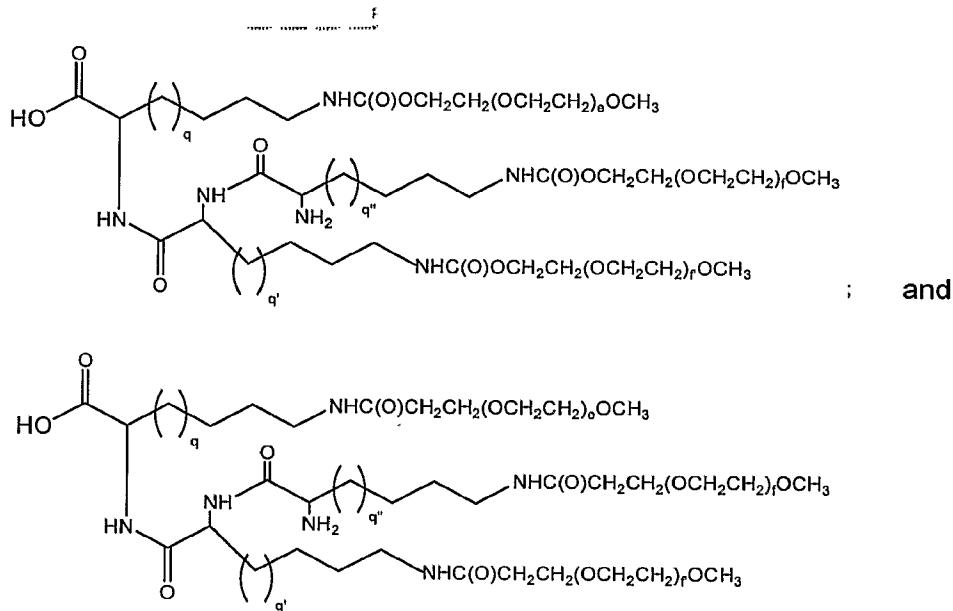
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[0175] Representative polymeric modifying moieties include structures that are based on side chain-containing amino acids, e.g., serine, cysteine, lysine, and small peptides, e.g., lys-20 lys. Exemplary structures include:



Those of skill will appreciate that the free amine in the di-lysine structures can also be pegylated through an amide or urethane bond with a PEG moiety.

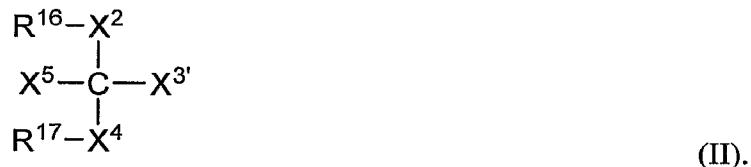
[0176] In yet another embodiment, the polymeric modifying moiety is a branched PEG moiety that is based upon a tri-lysine peptide. The tri-lysine can be mono-, di-, tri-, or tetra-PEG-ylated. Exemplary species according to this embodiment have the formulae:



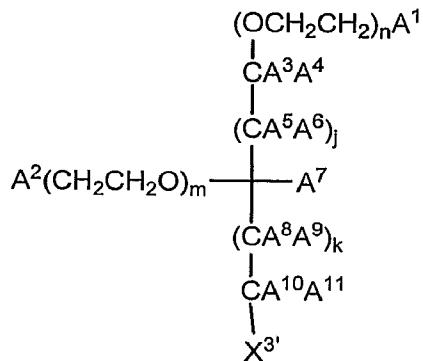
in which the indices e, f and f' are independently selected integers from 1 to 2500; and the indices q, q' and q'' are independently selected integers from 1 to 20.

**[0177]** As will be apparent to those of skill, the branched polymers of use in the invention include variations on the themes set forth above. For example the di-lysine-PEG conjugate shown above can include three polymeric subunits, the third bonded to the  $\alpha$ -amine shown as unmodified in the structure above. Similarly, the use of a tri-lysine functionalized with three or four polymeric subunits labeled with the polymeric modifying moiety in a desired manner is within the scope of the invention.

**[0178]** As discussed herein, the PEG of use in the conjugates of the invention can be linear or branched. An exemplary precursor of use to form the branched PEG containing peptide conjugates according to this embodiment of the invention has the formula:



Another exemplary precursor of use to form the branched PEG containing peptide conjugates according to this embodiment of the invention has the formula:



in which the indices m and n are integers independently selected from 0 to 5000. The indices t and a are independently selected from 0 or 1. The indices j and k are integers independently selected from 0 to 20. A<sup>1</sup>, A<sup>2</sup>, A<sup>3</sup>, A<sup>4</sup>, A<sup>5</sup>, A<sup>6</sup>, A<sup>7</sup>, A<sup>8</sup>, A<sup>9</sup>, A<sup>10</sup> and A<sup>11</sup> are members

5 independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, -NA<sup>12</sup>A<sup>13</sup>, -OA<sup>12</sup> and -SiA<sup>12</sup>A<sup>13</sup>. A<sup>12</sup> and A<sup>13</sup> are members independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or  
10 unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

**[0179]** The branched polymer species according to this formula are essentially pure water-soluble polymers. X<sup>3'</sup> is a moiety that includes an ionizable (*e.g.*, OH, COOH, H<sub>2</sub>PO<sub>4</sub>, HSO<sub>3</sub>, NH<sub>2</sub>, and salts thereof, etc.) or other reactive functional group, *e.g.*, *infra*. C is carbon.

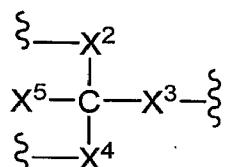
15 X<sup>5</sup>, R<sup>16</sup> and R<sup>17</sup> are independently selected from non-reactive groups (*e.g.*, H, unsubstituted alkyl, unsubstituted heteroalkyl) and polymeric arms (*e.g.*, PEG). X<sup>2</sup> and X<sup>4</sup> are linkage fragments that are preferably essentially non-reactive under physiological conditions, which may be the same or different. An exemplary linker includes neither aromatic nor ester  
20 moieties. Alternatively, these linkages can include one or more moiety that is designed to degrade under physiologically relevant conditions, *e.g.*, esters, disulfides, etc. X<sup>2</sup> and X<sup>4</sup> join polymeric arms R<sup>16</sup> and R<sup>17</sup> to C. When X<sup>3'</sup> is reacted with a reactive functional group of complementary reactivity on a linker, sugar or linker-sugar cassette, X<sup>3'</sup> is converted to a component of linkage fragment X<sup>3</sup>.

**[0180]** Exemplary linkage fragments for X<sup>2</sup>, X<sup>3</sup> and X<sup>4</sup> are independently selected and  
25 include S, SC(O)NH, HNC(O)S, SC(O)O, O, NH, NHC(O), (O)CNH and NHC(O)O, and OC(O)NH, CH<sub>2</sub>S, CH<sub>2</sub>O, CH<sub>2</sub>CH<sub>2</sub>O, CH<sub>2</sub>CH<sub>2</sub>S, (CH<sub>2</sub>)<sub>2</sub>O, (CH<sub>2</sub>)<sub>2</sub>S or (CH<sub>2</sub>)<sub>2</sub>Y'-PEG

wherein, Y' is S, NH, NHC(O), C(O)NH, NHC(O)O, OC(O)NH, or O and o is an integer from 1 to 50. In an exemplary embodiment, the linkage fragments X<sup>2</sup> and X<sup>4</sup> are different linkage fragments.

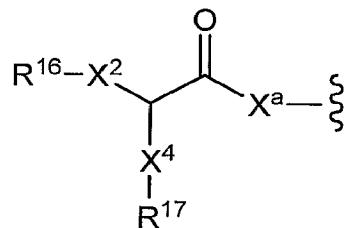
**[0181]** In an exemplary embodiment, the precursor (Formula II), or an activated derivative thereof, is reacted with, and thereby bound to a sugar, an activated sugar or a sugar nucleotide through a reaction between X<sup>3'</sup> and a group of complementary reactivity on the sugar moiety, e.g., an amine. Alternatively, X<sup>3'</sup> reacts with a reactive functional group on a precursor to linker, L.

**[0182]** In an exemplary embodiment, the moiety:



10

is the linker arm, L. In this embodiment, an exemplary linker is derived from a natural or unnatural amino acid, amino acid analogue or amino acid mimetic, or a small peptide formed from one or more such species. For example, certain branched polymers found in the compounds of the invention have the formula:

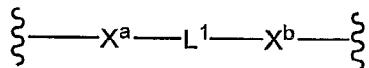


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(III)

**[0183]** X<sup>a</sup> is a linkage fragment that is formed by the reaction of a reactive functional group, e.g., X<sup>3'</sup>, on a precursor of the branched polymeric modifying moiety and a reactive functional group on the sugar moiety, or a precursor to a linker. For example, when X<sup>3'</sup> is a carboxylic acid, it can be activated and bound directly to an amine group pendent from an amino-saccharide (e.g., Sia, GalNH<sub>2</sub>, GlcNH<sub>2</sub>, ManNH<sub>2</sub>, etc.), forming a X<sup>a</sup> that is an amide. Additional exemplary reactive functional groups and activated precursors are described hereinbelow. The index c represents an integer from 1 to 10. The other symbols have the same identity as those discussed above.

**[0184]** In another exemplary embodiment, X<sup>a</sup> is a linking moiety formed with another linker:

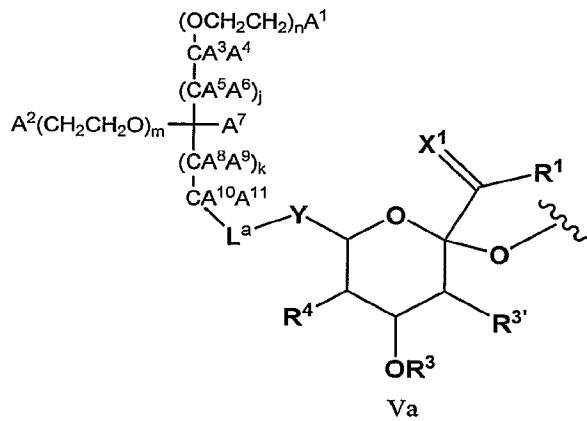
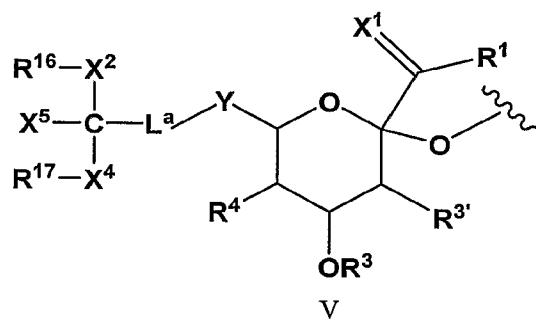


in which  $X^b$  is a second linkage fragment and is independently selected from those groups set forth for  $X^a$ , and, similar to  $L^a$ ,  $L^1$  is a bond, substituted or unsubstituted alkyl or substituted or unsubstituted heteroalkyl.

5 [0185] Exemplary species for  $X^a$  and  $X^b$  include S, SC(O)NH, HNC(O)S, SC(O)O, O, NH, NHC(O), C(O)NH and NHC(O)O, and OC(O)NH.

[0186] In another exemplary embodiment,  $X^4$  is a peptide bond to  $R^{17}$ , which is an amino acid, di-peptide (*e.g.*, Lys-Lys) or tri-peptide (*e.g.*, Lys-Lys-Lys) in which the alpha-amine moiety(ies) and/or side chain heteroatom(s) are modified with a polymeric modifying moiety.

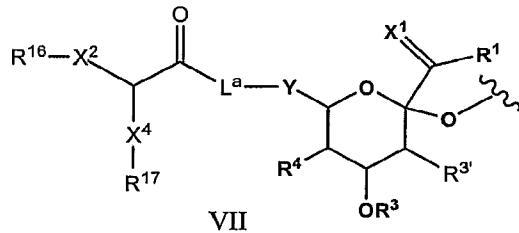
10 [0187] In a further exemplary embodiment, the peptide conjugates of the invention include a moiety, *e.g.*, an  $R^{15'}$  moiety that has a formula that is selected from:



15 in which the identity of the radicals represented by the various symbols is the same as that discussed hereinabove.  $L^a$  is a bond or a linker as discussed above for  $L$  and  $L^1$ , *e.g.*, substituted or unsubstituted alkyl or substituted or unsubstituted heteroalkyl moiety. In an exemplary embodiment,  $L^a$  is a moiety that is functionalized with the polymeric modifying moiety as shown. Exemplary  $L^a$  moieties include substituted or unsubstituted alkyl chains,

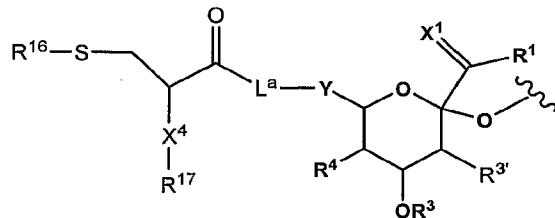
NH and NR<sup>6</sup>.

**[0188]** In yet another exemplary embodiment, the invention provides peptide conjugates having a moiety, *e.g.*, an R<sup>15'</sup> moiety with formula:



5 The identity of the radicals represented by the various symbols is the same as that discussed hereinabove. As those of skill will appreciate, the linker arm in Formula VII is equally applicable to other modified sugars set forth herein. In an exemplary embodiment, the species of Formula VII is the R<sup>15'</sup> moieties attached to the glycan structures set forth herein.

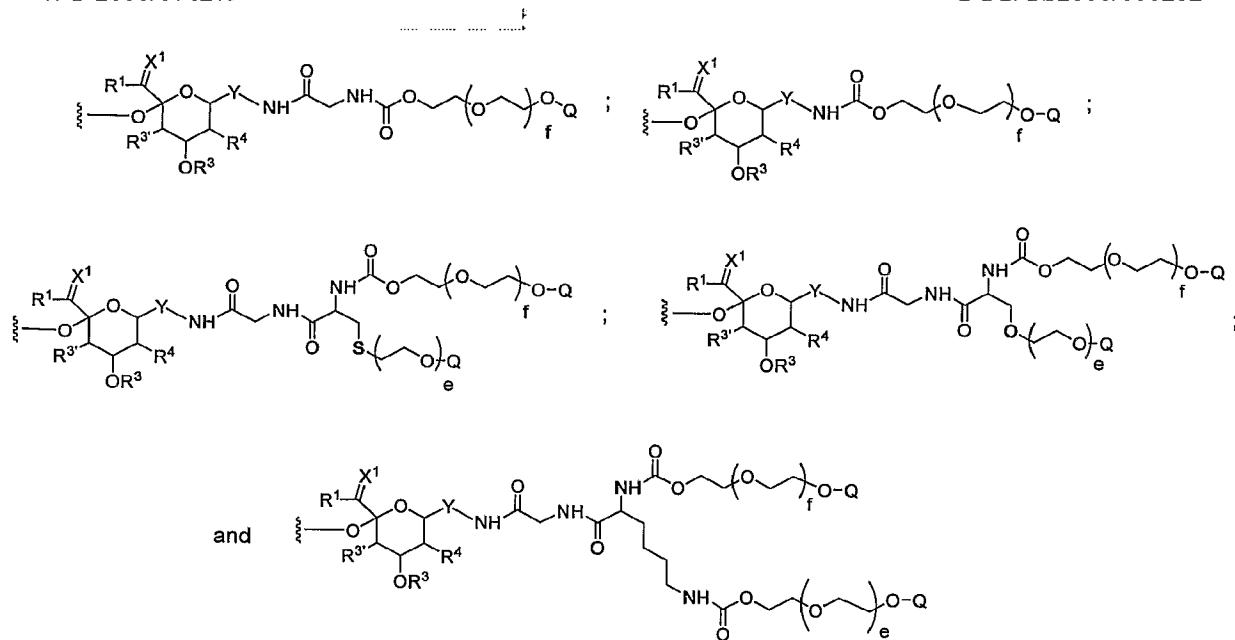
10 **[0189]** In an exemplary embodiment, the glycosyl linking group has a structure according to the following formula:



15 **[0190]** The embodiments of the invention set forth above are further exemplified by reference to species in which the polymer is a water-soluble polymer, particularly poly(ethylene glycol) (“PEG”), *e.g.*, methoxy-poly(ethylene glycol). Those of skill will appreciate that the focus in the sections that follow is for clarity of illustration and the various motifs set forth using PEG as an exemplary polymer are equally applicable to species in which a polymer other than PEG is utilized.

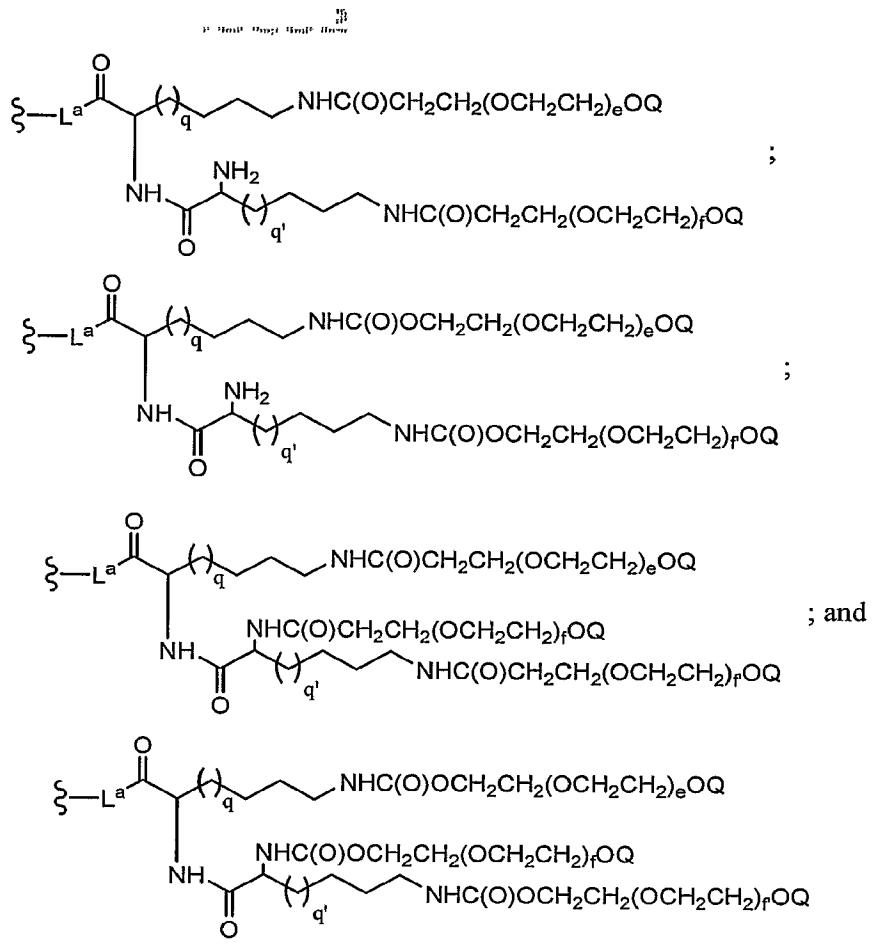
20 **[0191]** PEG of any molecular weight, *e.g.*, 1 kDa, 2 kDa, 5 kDa, 10 kDa, 15 kDa, 20 kDa, 25 kDa, 30 kDa, 35 kDa, 40 kDa, 45 kDa, 50 kDa, 55 kDa, 60 kDa, 65 kDa, 70 kDa, 75 kDa and 80 kDa is of use in the present invention.

**[0192]** In other exemplary embodiments, the peptide conjugate includes an R<sup>15'</sup> moiety selected from the group:

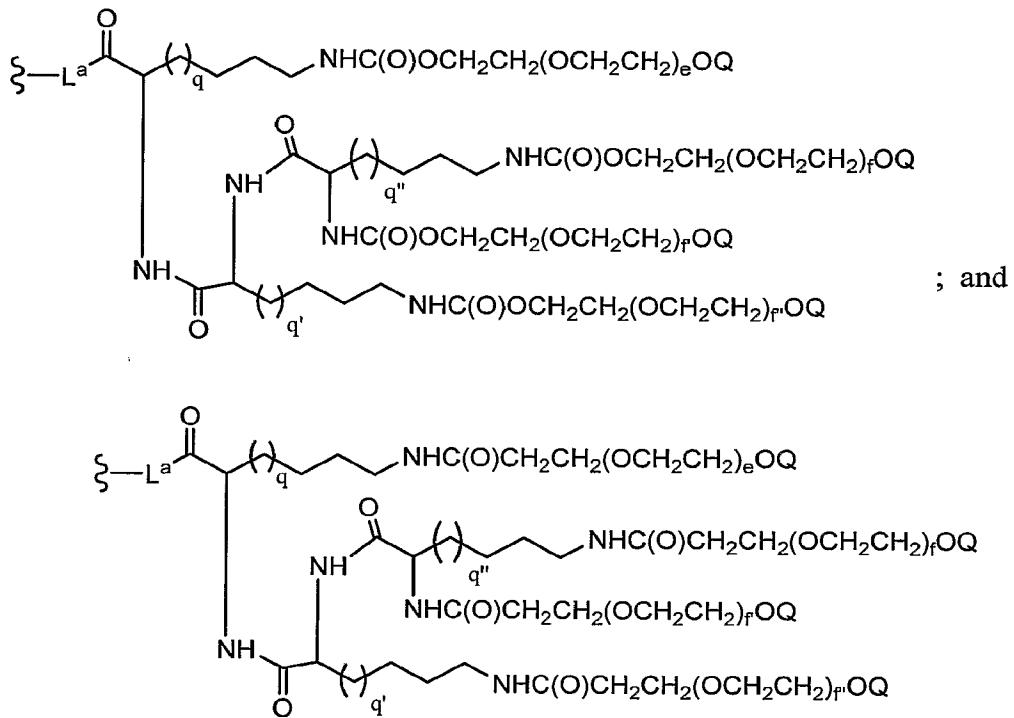


**[0193]** In each of the formulae above, the indices e and f are independently selected from the integers from 1 to 2500. In further exemplary embodiments, e and f are selected to provide a PEG moiety that is about 1 kDa, 2 kDa, 5 kDa, 10 kDa, 15 kDa, 20 kDa, 25 kDa, 5 30 kDa, 35 kDa, 40 kDa, 45 kDa, 50 kDa, 55 kDa, 60 kDa, 65 kDa, 70 kDa, 75 kDa and 80 kDa. The symbol Q represents substituted or unsubstituted alkyl (e.g., C<sub>1</sub>-C<sub>6</sub> alkyl, e.g., methyl), substituted or unsubstituted heteroalkyl or H.

**[0194]** Other branched polymers have structures based on di-lysine (Lys-Lys) peptides, e.g.:



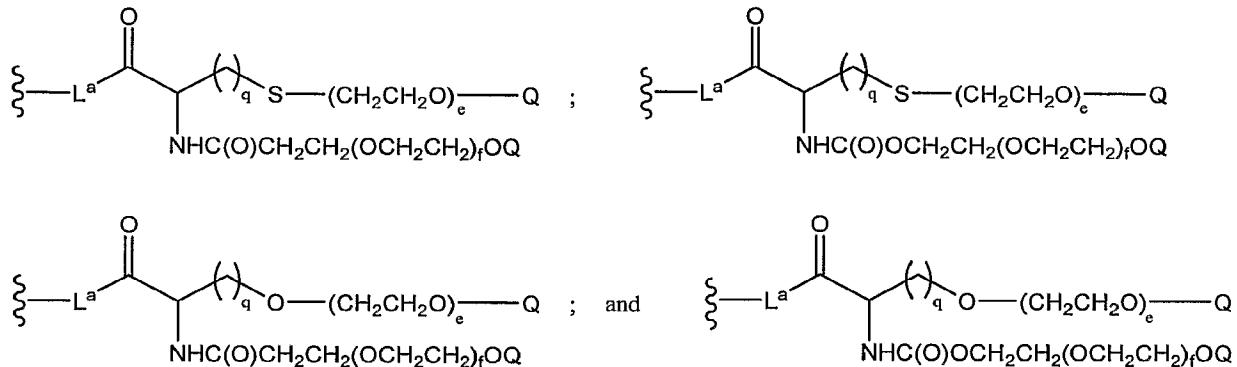
and tri-lysine peptides (Lys-Lys-Lys), e.g.:



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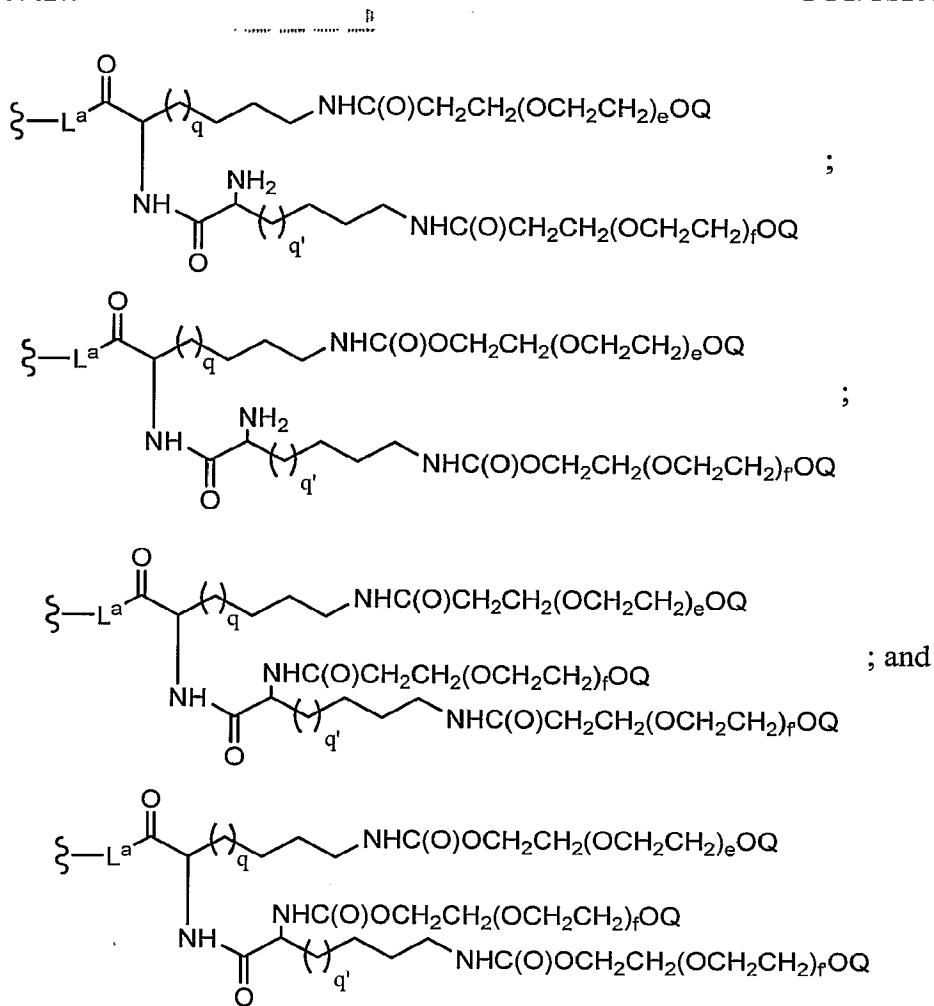
In each of the figures above, the indices e, f, f' and f'' represent integers independently selected from 1 to 2500. The indices q, q' and q'' represent integers independently selected from 1 to 20.

5 [0195] In another exemplary embodiment, Y<sup>2</sup> has a formula that is a member selected from:



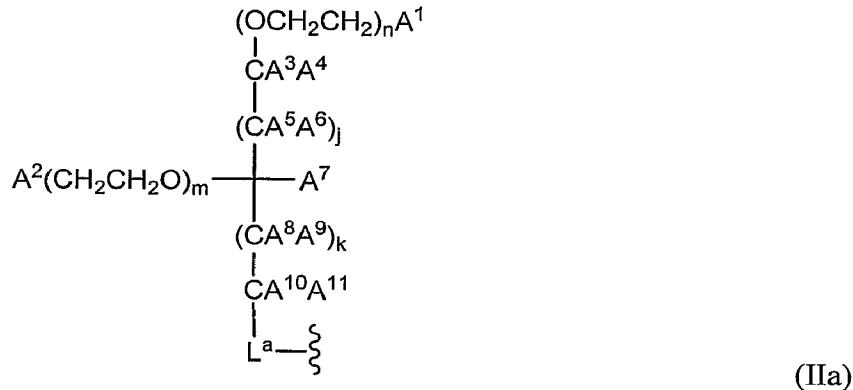
wherein Q is a member selected from H and substituted or unsubstituted C<sub>1</sub>-C<sub>6</sub> alkyl. The indices e and f are integers independently selected from 1 to 2500, and the index q is an integer selected from 0 to 20.

10 [0196] In another exemplary embodiment, Y<sup>2</sup> has a formula that is a member selected from:



wherein Q is a member selected from H and substituted or unsubstituted C<sub>1</sub>-C<sub>6</sub> alkyl. The indices e, f and f' are integers independently selected from 1 to 2500, and q and q' are integers independently selected from 1 to 20.

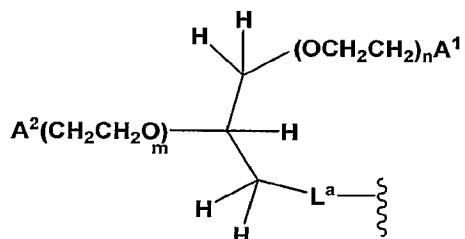
5 [0197] In another exemplary embodiment, the branched polymer has a structure according to the following formula:



in which the indices m and n are integers independently selected from 0 to 5000. The indices t and a are independently selected from 0 or 1. The indices j and k are integers independently selected from 0 to 20. A<sup>1</sup>, A<sup>2</sup>, A<sup>3</sup>, A<sup>4</sup>, A<sup>5</sup>, A<sup>6</sup>, A<sup>7</sup>, A<sup>8</sup>, A<sup>9</sup>, A<sup>10</sup> and A<sup>11</sup> are members independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, -NA<sup>12</sup>A<sup>13</sup>, -OA<sup>12</sup> and -SiA<sup>12</sup>A<sup>13</sup>. A<sup>12</sup> and A<sup>13</sup> are members independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

**[0198]** Formula IIa is a subset of Formula II. The structures described by Formula IIa are also encompassed by Formula II.

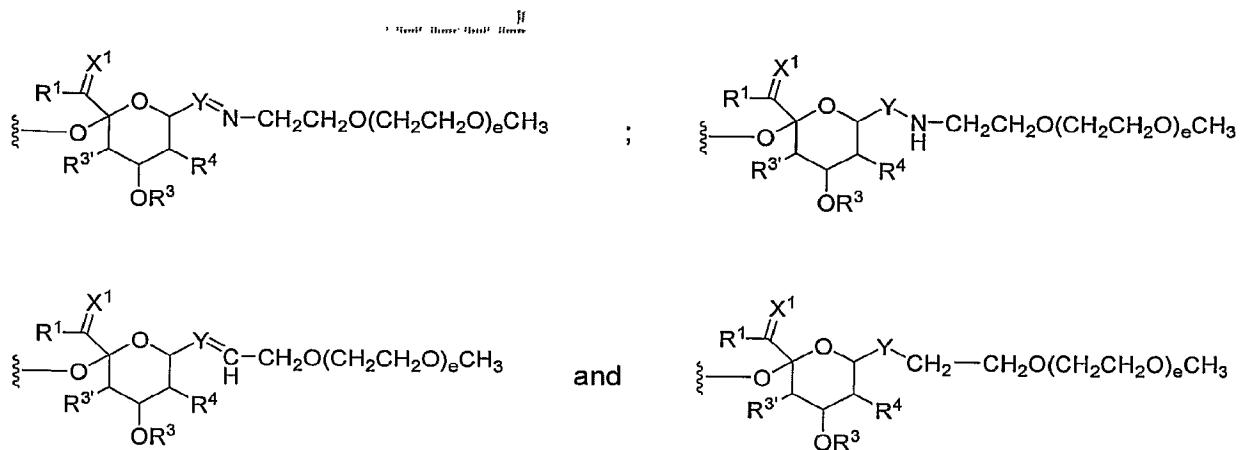
**[0199]** In another exemplary embodiment according to the formula above, the branched polymer has a structure according to the following formula:



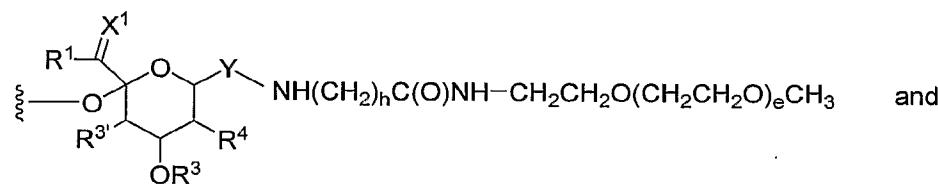
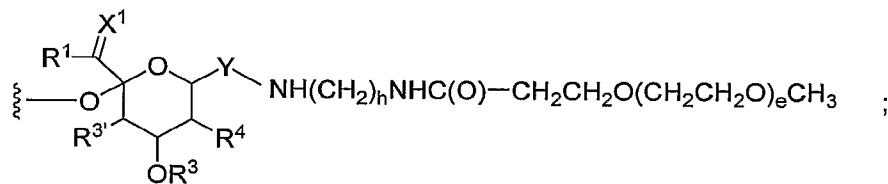
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In an exemplary embodiment, A<sup>1</sup> and A<sup>2</sup> are each -OCH<sub>3</sub> or H.

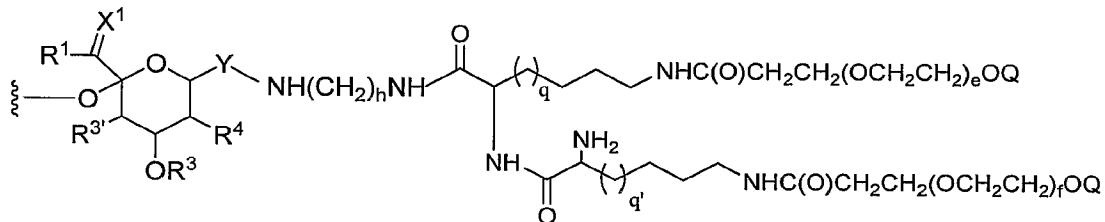
**[0200]** In an exemplary embodiment the modified saccharyl fragment is linked to the polymeric modifying group by reacting the aldehyde group of the oxidized sialyl side chain with a Grignard reagent or a Wittig reagent or an appropriate amine containing reagent, thereby forming an imine, which is alternatively reduced. Formulae according to this embodiment include:



**[0201]** In another exemplary embodiment the modified saccharyl fragment is linked to the polymeric modifying group through a diamino alkyl linker or an amino carboxylic acid linker. Formulae according to this embodiment include:

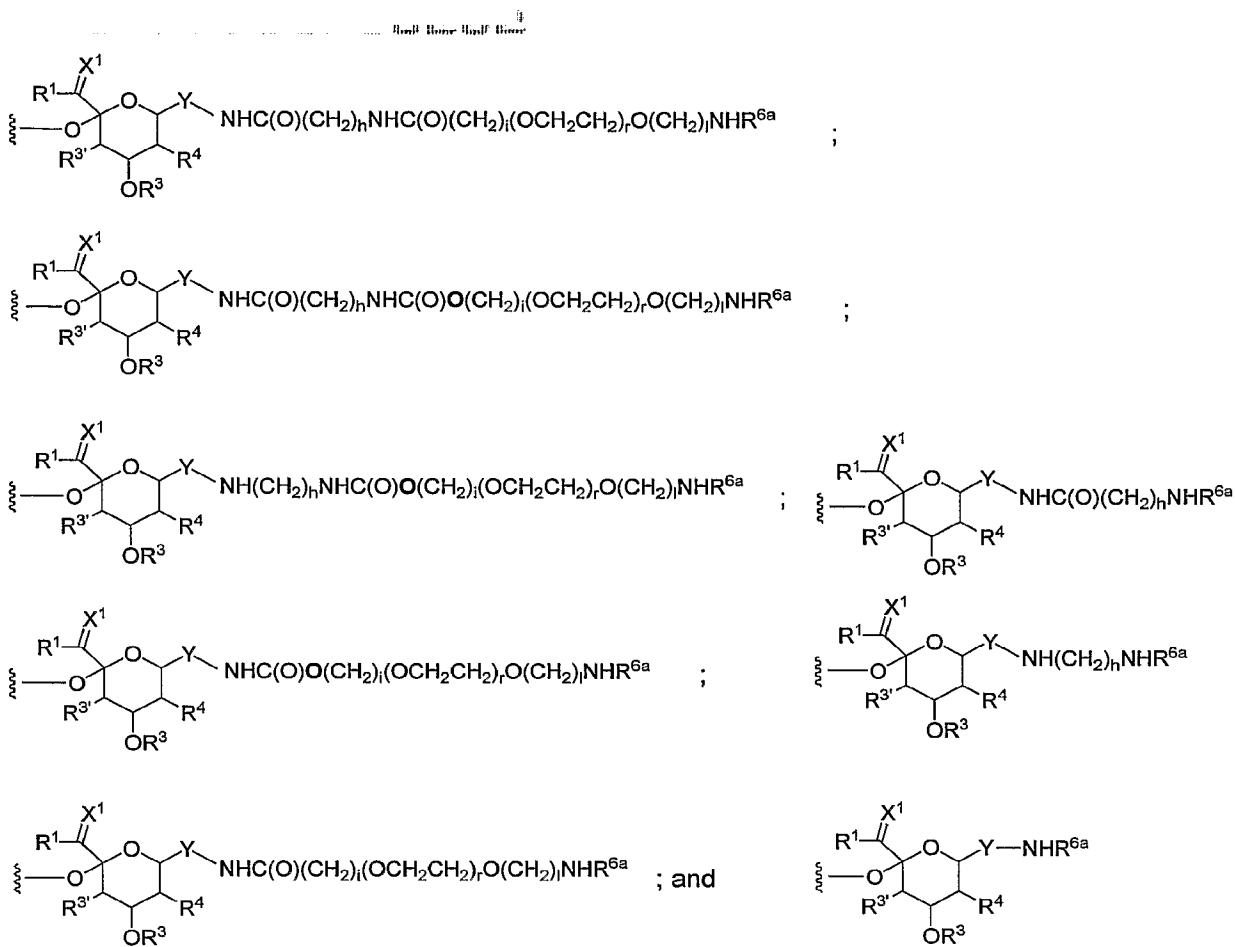


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in which the index  $h$  is an integer from 0 to 20 and the indices  $q$ ,  $q'$ ,  $e$  and  $f$  are as defined above.

**[0202]** In an illustrative embodiment, the aldehyde group of the oxidized sialyl side chain 10 of the modified saccharyl fragment is functionalized with the modifying group. For example, the aldehyde is reductively aminated with ammonia. The resulting primary amine is functionalized to provide a compound according to the invention. Formulae according to this embodiment include:



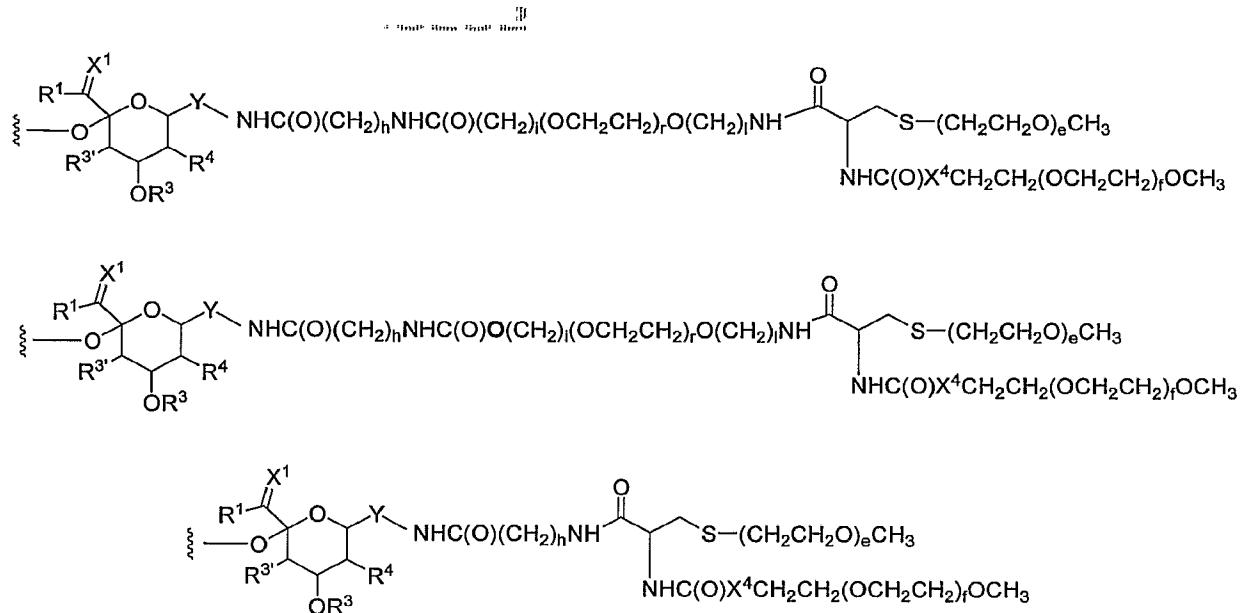
The indices  $h$ ,  $i$  and  $l$  are integers from 0 to 20. The index  $r$  is an integer from 1 to 2500. The structures set forth above can be components of  $R^{15'}$ .

[0203] Although the present invention is exemplified in the preceding sections by

5 reference to PEG, as those of skill will appreciate, an array of polymeric modifying moieties  
is of use in the compounds and methods set forth herein.

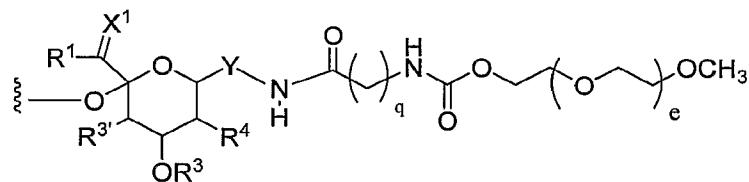
[0204] In selected embodiments, R<sup>6a</sup> or L- R<sup>6b</sup> is a branched PEG, for example, one of the species set forth above. In an exemplary embodiment, the branched PEG structure is based on a cysteine peptide. Illustrative modified saccharyl fragments according to this

10 embodiment include:



in which X<sup>4</sup> is a bond or O. In each of the structures above, the alkylamine linker – NHC(O)(CH<sub>2</sub>)<sub>n</sub>- can be present or absent. The structures set forth above can be components of R<sup>15</sup>/R<sup>15'</sup>.

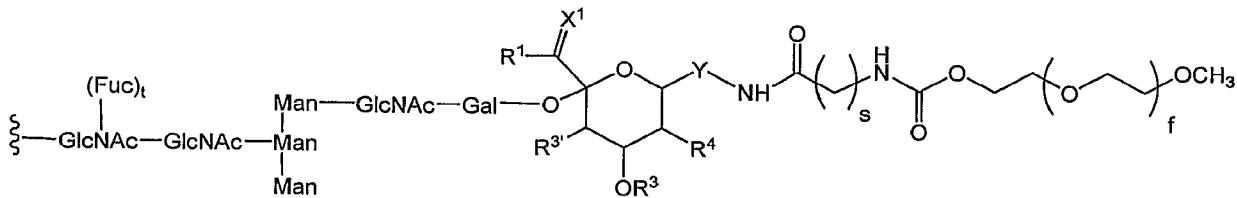
5 [0205] As discussed herein, the polymeric modifying groups of use in the invention may also be linear structures. Thus, the invention provides for conjugates that include a modified saccharyl fragment derived from a structure such as:



in which the indices q and e are as discussed above.

10 [0206] Exemplary modified sugars are modified with water-soluble or water-insoluble polymers. Examples of useful polymer are further exemplified below.

[0207] In another exemplary embodiment, the peptide is derived from insect cells, remodeled by adding GlcNAc and Gal to the mannose core and glycopegylated using a sialic acid bearing a linear PEG moiety, affording a peptide conjugate that comprises at least one moiety having the formula:



in which the index t is an integer from 0 to 1; the index s represents an integer from 1 to 10; and the index f represents an integer from 1 to 2500.

### Water-Insoluble Polymers

5 [0208] In another embodiment, analogous to those discussed above, the modified sugars include a water-insoluble polymer, rather than a water-soluble polymer. The conjugates of the invention may also include one or more water-insoluble polymers. This embodiment of the invention is illustrated by the use of the conjugate as a vehicle with which to deliver a therapeutic peptide in a controlled manner. Polymeric drug delivery systems are known in  
10 the art. See, for example, Dunn *et al.*, Eds. POLYMERIC DRUGS AND DRUG DELIVERY SYSTEMS, ACS Symposium Series Vol. 469, American Chemical Society, Washington, D.C. 1991. Those of skill in the art will appreciate that substantially any known drug delivery system is applicable to the conjugates of the present invention.

15 [0209] The motifs set forth above for  $R^{6a}$ ,  $L^a-R^{6a}$ ,  $R^{15}$ ,  $R^{15'}$  and other radicals are equally applicable to water-insoluble polymers, which may be incorporated into the linear and branched structures without limitation utilizing chemistry readily accessible to those of skill in the art. Similarly, the incorporation of these species into any of the modified sugars discussed herein is within the scope of the present invention. Accordingly, the invention provides conjugates containing, and for the use of to prepare such conjugates, sialic acid and  
20 other sugar moieties modified with a linear or branched water-insoluble polymers, and activated analogues of the modified sialic acid species (*e.g.*, CMP-Sia-(water insoluble polymer)).

25 [0210] Representative water-insoluble polymers include, but are not limited to, polyphosphazines, poly(vinyl alcohols), polyamides, polycarbonates, polyalkylenes, polyacrylamides, polyalkylene glycols, polyalkylene oxides, polyalkylene terephthalates, polyvinyl ethers, polyvinyl esters, polyvinyl halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes, poly(methyl methacrylate), poly(ethyl methacrylate), poly(butyl methacrylate), poly(isobutyl methacrylate), poly(hexyl methacrylate),

poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) polyethylene, polypropylene, poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl acetate), polyvinyl chloride, polystyrene, polyvinyl pyrrolidone, pluronic and polyvinylphenol and copolymers thereof.

[0211] Synthetically modified natural polymers of use in conjugates of the invention include, but are not limited to, alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, and nitrocelluloses. Particularly preferred members of the broad classes of synthetically modified natural polymers include, but are not limited to, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxymethyl cellulose, cellulose triacetate, cellulose sulfate sodium salt, and polymers of acrylic and methacrylic esters and alginic acid.

[0212] These and the other polymers discussed herein can be readily obtained from commercial sources such as Sigma Chemical Co. (St. Louis, MO.), Polysciences (Warrenton, PA.), Aldrich (Milwaukee, WI), Fluka (Ronkonkoma, NY), and BioRad (Richmond, CA), or else synthesized from monomers obtained from these suppliers using standard techniques.

[0213] Representative biodegradable polymers of use in the conjugates of the invention include, but are not limited to, polylactides, polyglycolides and copolymers thereof, poly(ethylene terephthalate), poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), poly(lactide-co-glycolide), polyanhydrides, polyorthoesters, blends and copolymers thereof. Of particular use are compositions that form gels, such as those including collagen, pluronic and the like.

[0214] The polymers of use in the invention include "hybrid" polymers that include water-insoluble materials having within at least a portion of their structure, a bioresorbable molecule. An example of such a polymer is one that includes a water-insoluble copolymer, which has a bioresorbable region, a hydrophilic region and a plurality of crosslinkable functional groups per polymer chain.

[0215] For purposes of the present invention, "water-insoluble materials" includes materials that are substantially insoluble in water or water-containing environments. Thus, although certain regions or segments of the copolymer may be hydrophilic or even water-

soluble, the polymer molecule, as a whole, does not to any substantial measure dissolve in water.

[0216] For purposes of the present invention, the term "bioresorbable molecule" includes a region that is capable of being metabolized or broken down and resorbed and/or eliminated through normal excretory routes by the body. Such metabolites or break down products are preferably substantially non-toxic to the body.

[0217] The bioresorbable region may be either hydrophobic or hydrophilic, so long as the copolymer composition as a whole is not rendered water-soluble. Thus, the bioresorbable region is selected based on the preference that the polymer, as a whole, remains water-insoluble. Accordingly, the relative properties, *i.e.*, the kinds of functional groups contained by, and the relative proportions of the bioresorbable region, and the hydrophilic region are selected to ensure that useful bioresorbable compositions remain water-insoluble.

[0218] Exemplary resorbable polymers include, for example, synthetically produced resorbable block copolymers of poly( $\alpha$ -hydroxy-carboxylic acid)/poly(oxyalkylene, (*see*, Cohn *et al.*, U.S. Patent No. 4,826,945). These copolymers are not crosslinked and are water-soluble so that the body can excrete the degraded block copolymer compositions. *See*, Younes *et al.*, *J Biomed. Mater. Res.* **21**: 1301-1316 (1987); and Cohn *et al.*, *J Biomed. Mater. Res.* **22**: 993-1009 (1988).

[0219] Presently preferred bioresorbable polymers include one or more components selected from poly(esters), poly(hydroxy acids), poly(lactones), poly(amides), poly(ester-amides), poly (amino acids), poly(anhydrides), poly(orthoesters), poly(carbonates), poly(phosphazines), poly(phosphoesters), poly(thioesters), polysaccharides and mixtures thereof. More preferably still, the biosresorbable polymer includes a poly(hydroxy) acid component. Of the poly(hydroxy) acids, polylactic acid, polyglycolic acid, polycaproic acid, polybutyric acid, polyvaleric acid and copolymers and mixtures thereof are preferred.

[0220] In addition to forming fragments that are absorbed *in vivo* ("bioresorbed"), preferred polymeric coatings for use in the methods of the invention can also form an excretable and/or metabolizable fragment.

[0221] Higher order copolymers can also be used in the present invention. For example, Casey *et al.*, U.S. Patent No. 4,438,253, which issued on March 20, 1984, discloses tri-block copolymers produced from the transesterification of poly(glycolic acid) and an hydroxyl-

ended poly(alkylene glycol). Such compositions are disclosed for use as resorbable monofilament sutures. The flexibility of such compositions is controlled by the incorporation of an aromatic orthocarbonate, such as tetra-p-tolyl orthocarbonate into the copolymer structure.

5 [0222] Other polymers based on lactic and/or glycolic acids can also be utilized. For example, Spinu, U.S. Patent No. 5,202,413, which issued on April 13, 1993, discloses biodegradable multi-block copolymers having sequentially ordered blocks of polylactide and/or polyglycolide produced by ring-opening polymerization of lactide and/or glycolide onto either an oligomeric diol or a diamine residue followed by chain extension with a di-  
10 functional compound, such as, a diisocyanate, diacylchloride or dichlorosilane.

[0223] Bioresorbable regions of coatings useful in the present invention can be designed to be hydrolytically and/or enzymatically cleavable. For purposes of the present invention, "hydrolytically cleavable" refers to the susceptibility of the copolymer, especially the bioresorbable region, to hydrolysis in water or a water-containing environment. Similarly, 15 "enzymatically cleavable" as used herein refers to the susceptibility of the copolymer, especially the bioresorbable region, to cleavage by endogenous or exogenous enzymes.

[0224] When placed within the body, the hydrophilic region can be processed into excretable and/or metabolizable fragments. Thus, the hydrophilic region can include, for example, polyethers, polyalkylene oxides, polyols, poly(vinyl pyrrolidine), poly(vinyl alcohol), poly(alkyl oxazolines), polysaccharides, carbohydrates, peptides, proteins and copolymers and mixtures thereof. Furthermore, the hydrophilic region can also be, for example, a poly(alkylene) oxide. Such poly(alkylene) oxides can include, for example, poly(ethylene) oxide, poly(propylene) oxide and mixtures and copolymers thereof.  
20

[0225] Polymers that are components of hydrogels are also useful in the present invention.  
25 Hydrogels are polymeric materials that are capable of absorbing relatively large quantities of water. Examples of hydrogel forming compounds include, but are not limited to, polyacrylic acids, sodium carboxymethylcellulose, polyvinyl alcohol, polyvinyl pyrrolidine, gelatin, carrageenan and other polysaccharides, hydroxyethylmethacrylic acid (HEMA), as well as derivatives thereof, and the like. Hydrogels can be produced that are stable, biodegradable  
30 and bioresorbable. Moreover, hydrogel compositions can include subunits that exhibit one or more of these properties.

[0226] Bio-compatible hydrogel compositions whose integrity can be controlled through crosslinking are known and are presently preferred for use in the methods of the invention. For example, Hubbell *et al.*, U.S. Patent Nos. 5,410,016, which issued on April 25, 1995 and 5,529,914, which issued on June 25, 1996, disclose water-soluble systems, which are

5 crosslinked block copolymers having a water-soluble central block segment sandwiched between two hydrolytically labile extensions. Such copolymers are further end-capped with photopolymerizable acrylate functionalities. When crosslinked, these systems become hydrogels. The water soluble central block of such copolymers can include poly(ethylene glycol); whereas, the hydrolytically labile extensions can be a poly( $\alpha$ -hydroxy acid), such as  
10 polyglycolic acid or polylactic acid. *See*, Sawhney *et al.*, *Macromolecules* 26: 581-587 (1993).

[0227] In another preferred embodiment, the gel is a thermoreversible gel. Thermoreversible gels including components, such as pluronic, collagen, gelatin, hyalouronic acid, polysaccharides, polyurethane hydrogel, polyurethane-urea hydrogel and  
15 combinations thereof are presently preferred.

[0228] In yet another exemplary embodiment, the conjugate of the invention includes a component of a liposome. Liposomes can be prepared according to methods known to those skilled in the art, for example, as described in Eppstein *et al.*, U.S. Patent No. 4,522,811. For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as  
20 stearoyl phosphatidyl ethanolamine, stearoyl phosphatidyl choline, arachadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the active compound or its pharmaceutically acceptable salt is then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to  
25 disperse lipid aggregates, thereby forming the liposomal suspension.

[0229] The above-recited microparticles and methods of preparing the microparticles are offered by way of example and they are not intended to define the scope of microparticles of use in the present invention. It will be apparent to those of skill in the art that an array of microparticles, fabricated by different methods, is of use in the present invention.

30 [0230] The structural formats discussed above in the context of the water-soluble polymers, both straight-chain and branched are generally applicable with respect to the water-insoluble polymers as well. Thus, for example, the cysteine, serine, diliysine, and trilysine

branching cores can be functionalized with two water-insoluble polymer moieties. The methods used to produce these species are generally closely analogous to those used to produce the water-soluble polymers.

Biomolecules

5 [0231] In another exemplary embodiment, the modified saccharyl fragment bears a biomolecule. In still further preferred embodiments, the biomolecule is a functional protein, enzyme, antigen, antibody, peptide, nucleic acid (*e.g.*, single nucleotides or nucleosides, oligonucleotides, polynucleotides and single- and higher-stranded nucleic acids), lectin, receptor or a combination thereof.

10 [0232] In a presently preferred embodiment, the modifying group is biotin. In an exemplary embodiment, the selectively biotinylated peptide is elaborated by the attachment of an avidin or streptavidin moiety bearing one or more modifying groups. Preferred biomolecules are essentially non-fluorescent, or emit such a minimal amount of fluorescence that they are inappropriate for use as a fluorescent marker in an assay. Moreover, it is

15 generally preferred to use biomolecules that are not sugars. An exception to this preference is the use of an otherwise naturally occurring sugar that is modified by covalent attachment of another entity (*e.g.*, PEG, biomolecule, therapeutic moiety, diagnostic moiety, *etc.*). In an exemplary embodiment, a sugar moiety, which is a biomolecule, is conjugated to a linker arm and the sugar-linker arm cassette is subsequently conjugated to a peptide via a method of the

20 invention.

[0233] Biomolecules useful in practicing the present invention can be derived from any source. The biomolecules can be isolated from natural sources or they can be produced by synthetic methods. Peptides can be natural peptides or mutated peptides. Mutations can be effected by chemical mutagenesis, site-directed mutagenesis or other means of inducing mutations known to those of skill in the art. Peptides useful in practicing the instant invention include, for example, enzymes, antigens, antibodies and receptors. Antibodies can be either polyclonal or monoclonal.

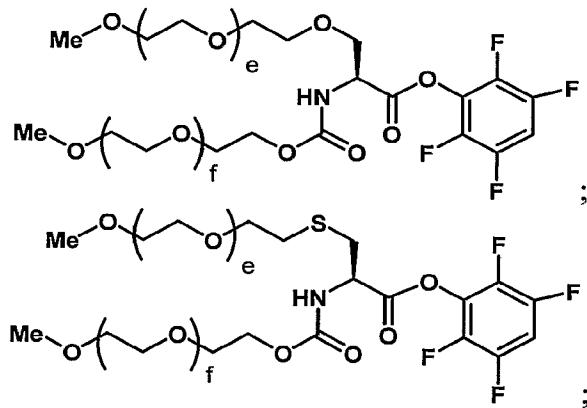
[0234] Both naturally derived and synthetic peptides and nucleic acids are of use in conjunction with the present invention; these molecules can be attached to a sugar residue component or a crosslinking agent by any available reactive group. For example, peptides can be attached through a reactive amine, carboxyl, sulphhydryl, or hydroxyl group. The reactive group can reside at a peptide terminus or at a site internal to the peptide chain.

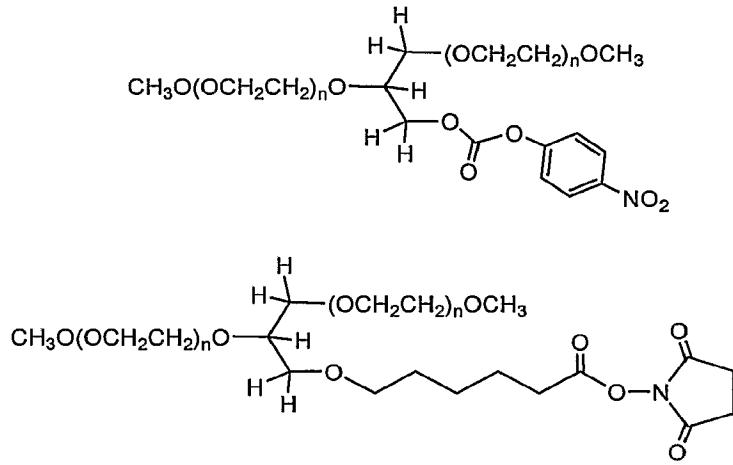
Nucleic acids can be attached through a reactive group on a base (e.g., exocyclic amine) or an available hydroxyl group on a sugar moiety (e.g., 3'- or 5'-hydroxyl). The peptide and nucleic acid chains can be further derivatized at one or more sites to allow for the attachment of appropriate reactive groups onto the chain. See, Chrisey *et al.* *Nucleic Acids Res.* **24:** 5 3031-3039 (1996).

[0235] In a further preferred embodiment, the biomolecule is selected to direct the peptide modified by the methods of the invention to a specific tissue, thereby enhancing the delivery of the peptide to that tissue relative to the amount of underderivatized peptide that is delivered to the tissue. In a still further preferred embodiment, the amount of derivatized peptide 10 delivered to a specific tissue within a selected time period is enhanced by derivatization by at least about 20%, more preferably, at least about 40%, and more preferably still, at least about 100%. Presently, preferred biomolecules for targeting applications include antibodies, hormones and ligands for cell-surface receptors.

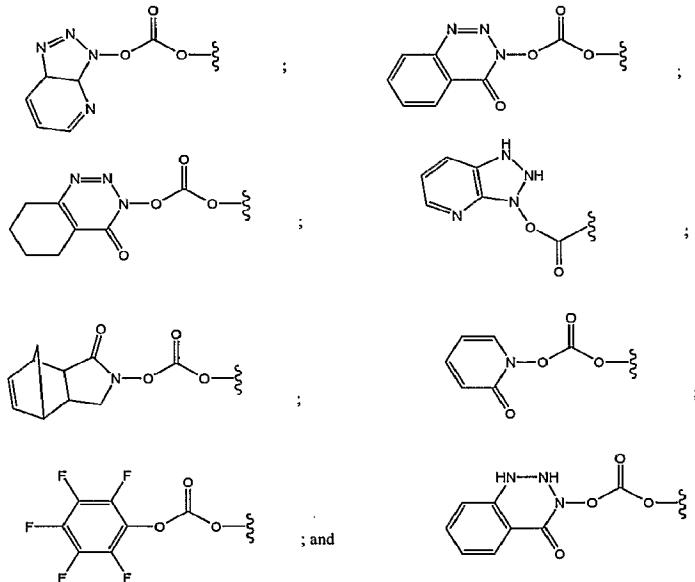
#### *II. D. v. Methods of Producing the Polymeric Modifying Groups*

[0236] The polymeric modifying groups can be activated for reaction with a glycosyl or saccharyl moiety, an amino acid moiety, an amine or with other nucleophiles. Exemplary structures of activated species (e.g., carbonates and active esters) include:





**[0237]** Other activating, or leaving groups, appropriate for activating linear and branched PEGs of use in preparing the compounds set forth herein include, but are not limited to the species:



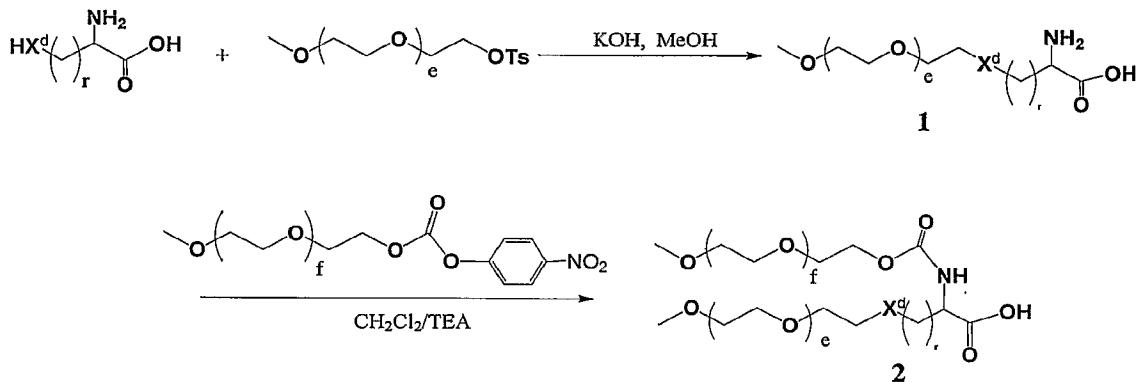
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PEG molecules that are activated with these and other species and methods of making the activated PEGs are set forth in WO 04/083259.

**[0238]** Those of skill in the art will appreciate that one or more of the m-PEG arms of the branched polymers shown above can be replaced by a PEG moiety with a different terminus, e.g., OH, COOH, NH<sub>2</sub>, C<sub>2</sub>-C<sub>10</sub>-alkyl, etc. Moreover, the structures above are readily modified by inserting alkyl linkers (or removing carbon atoms) between the α-carbon atom and the functional group of the amino acid side chain. Thus, “homo” derivatives and higher

homologues, as well as lower homologues are within the scope of cores for branched PEGs of use in the present invention.

**[0239]** The branched PEG species set forth herein are readily prepared by methods such as that set forth in the scheme below:

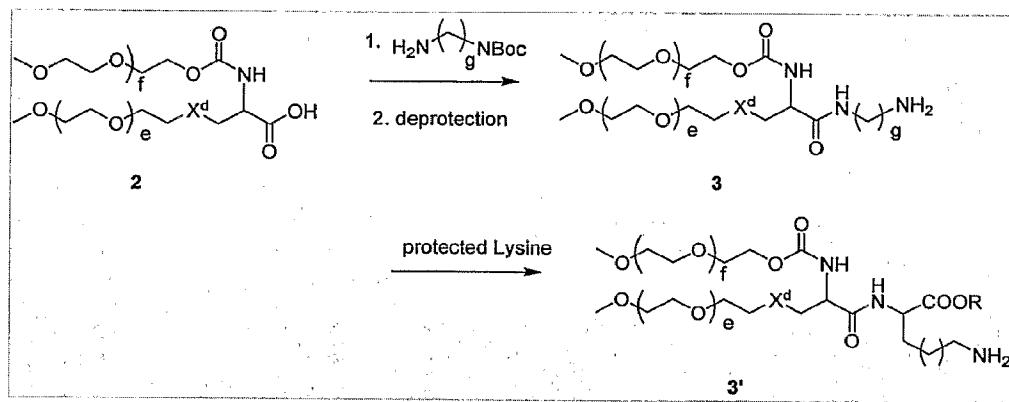


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in which  $X^d$  is O or S and r is an integer from 1 to 5. The indices e and f are independently selected integers from 1 to 2500. In an exemplary embodiment, one or both of these indices are selected such that the polymer is about 1 kDa, 2 kDa, 5 kDa, 10 kDa, 15 kDa, 20 kDa, 25 kDa, 30 kDa, 35 kDa, 40 kDa, 45 kDa, 50 kDa, 55 kDa, 60 kDa, 65 kDa, 70 kDa, 75 kDa or 10 80 kDa in molecular weight.

**[0240]** Thus, according to this scheme, a natural or unnatural amino acid is contacted with an activated m-PEG derivative, in this case the tosylate, forming 1 by alkylating the side-chain heteroatom  $X^d$ . The mono-functionalized m-PEG amino acid is submitted to N-acylation conditions with a reactive m-PEG derivative, thereby assembling branched m-PEG 15 2. As one of skill will appreciate, the tosylate leaving group can be replaced with any suitable leaving group, e.g., halogen, mesylate, triflate, etc. Similarly, the reactive carbonate utilized to acylate the amine can be replaced with an active ester, e.g., N-hydroxysuccinimide, etc., or the acid can be activated *in situ* using a dehydrating agent such as dicyclohexylcarbodiimide, carbonyldiimidazole, etc.

20 **[0241]** In other exemplary embodiments, the urea moiety is replaced by a group such as an amide.



### II. E. Homodisperse Peptide Conjugate Compositions of Matter

[0242] In addition to providing peptide conjugates that are formed through a chemically or enzymatically added glycosyl linking group, the present invention provides compositions 5 of matter comprising peptide conjugates that are highly homogenous in their substitution patterns. Using the methods of the invention, it is possible to form peptide conjugates in which substantial proportion of the glycosyl linking groups and glycosyl moieties across a population of peptide conjugates are attached to a structurally identical amino acid or glycosyl residue. Thus, in another aspect, the invention provides a peptide conjugate having 10 a population of water-soluble polymer moieties, which are covalently bound to the peptide through a glycosyl linking group, e.g., a modified saccharyl fragment. In an exemplary peptide conjugate of the invention, essentially each member of the water soluble polymer population is bound via the modified saccharyl fragment to a glycosyl residue of the peptide, and each glycosyl residue of the peptide to which the modified saccharyl fragment is attached 15 has the same structure.

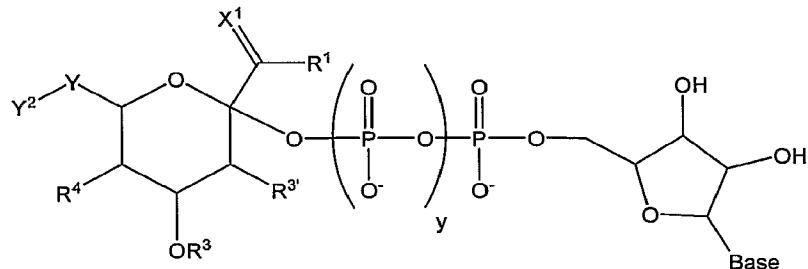
[0243] The present invention also provides conjugates analogous to those described above in which the peptide is conjugated to a modifying group, e.g. therapeutic moiety, diagnostic moiety, targeting moiety, toxin moiety or the like via a glycosyl linking group such as a modified saccharyl fragment. Each of the above-recited modifying groups can be a 20 small molecule, natural polymer (e.g., polypeptide) or synthetic polymer. When the modifying group is attached to a sialic acid, it is generally preferred that the modifying group is substantially non-fluorescent.

[0244] In an exemplary embodiment, the peptides of the invention include at least one O-linked or N-linked glycosylation site, which is glycosylated with a modified sugar that 25 includes a polymeric modifying group, e.g., a PEG moiety. In an exemplary embodiment, the

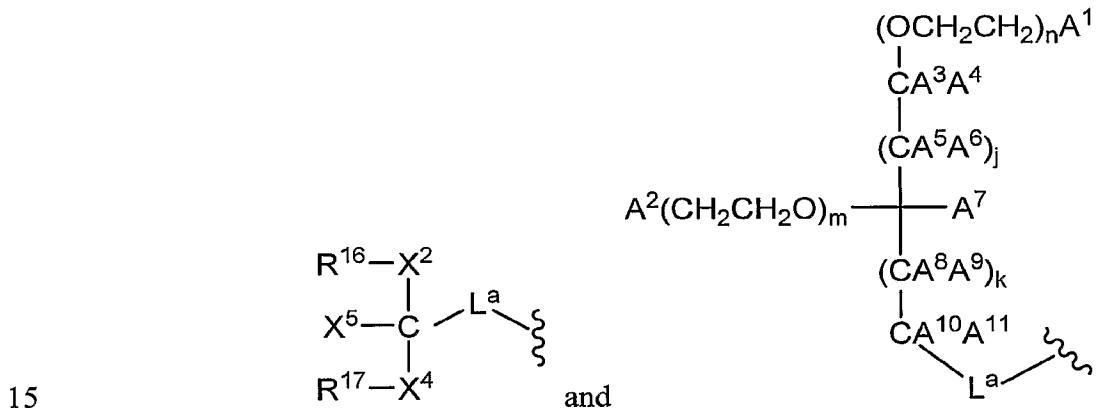
PEG is covalently attached to the peptide via an intact glycosyl linking group such as a modified saccharyl fragment, or via a non-glycosyl linker, e.g., substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl. The glycosyl linking group is covalently attached to either an amino acid residue or a glycosyl residue of the peptide. Alternatively, 5 the glycosyl linking group is attached to one or more glycosyl units of a glycopeptide. The invention also provides conjugates in which a glycosyl linking group is attached to both an amino acid residue and a glycosyl residue.

## ***II. F. Nucleotide Sugars***

[0245] In another aspect of the invention, the invention also provides sugar nucleotides. 10 Exemplary species according to this embodiment include:



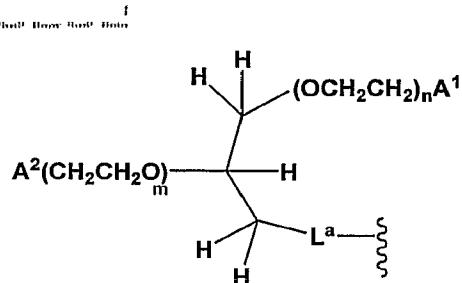
in which the index y is an integer selected from 0, 1 and 2. Base is a nucleic acid base, such as adenine, thymine, guanine, cytidine and uridine. Y<sup>2</sup>, X<sup>1</sup>, Y<sup>2</sup>, R<sup>1</sup>, R<sup>3</sup> and R<sup>4</sup> are as described above. In an exemplary embodiment, Y<sup>2</sup> or L<sup>a</sup>-(R<sup>6a</sup>)<sub>w</sub> is a member selected from



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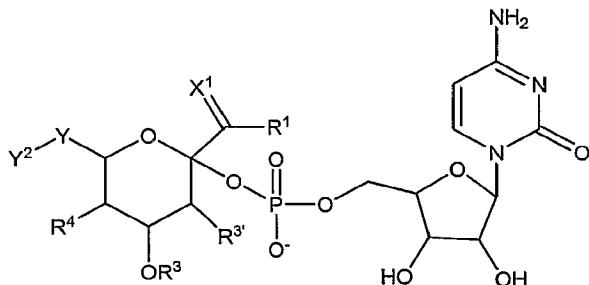
in which the variables are as described above.

[0246] In an exemplary embodiment, Y<sup>2</sup> or L<sup>a</sup>-(R<sup>6a</sup>)<sub>w</sub> has a structure according to the following formula:



In an exemplary embodiment, A<sup>1</sup> and A<sup>2</sup> are each -OCH<sub>3</sub>.

**[0247]** In another exemplary embodiment, the nucleotide sugar has a structure according to the following formula:



5

### The Methods

**[0248]** In addition to the compositions discussed above, the present invention provides methods for preparing modified saccharyl fragments and glyco-conjugates incorporating these fragments. Exemplary methods include synthesizing a modified peptide or lipid using a modified saccharyl fragment, *e.g.*, modified-galactose, -fucose, and -sialic acid. When a modified sialic acid is used, either a sialyltransferase or a trans-sialidase (for  $\alpha$ 2,3-linked sialic acid only) can be used to transfer the modified fragment onto the acceptor moiety of the substrate.

**[0249]** The method of the invention includes transferring a modified saccharyl fragment from an activated modified saccharyl fragment onto an acceptor moiety of a substrate. Exemplary substrates include peptides and lipids of therapeutic relevance. Exemplary acceptor moieties include amino acid residues, aglycone residues and glycosyl moieties directly or indirectly bound to an amino acid or aglycone residue.

**[0250]** For clarity of illustration, the invention is illustrated with reference to a conjugate formed between a (glyco)peptide a modified saccharyl fragment that is transferred to an acceptor moiety on the (glyco)peptide from an activated modified saccharyl fragment that includes a water-soluble polymer. Those of skill will appreciate that the invention equally

encompasses methods of forming conjugates of (glyco)lipids with saccharyl fragments modified with water-soluble polymers, and forming conjugates between (glyco)peptides and (glyco)lipids and saccharyl fragments bearing modifying groups other than water-soluble polymers.

5 [0251] In exemplary embodiments, the conjugate is formed between a water-soluble polymer, a therapeutic moiety, targeting moiety or a biomolecule, and a glycosylated peptide. The polymer, therapeutic moiety or biomolecule is conjugated to the peptide via a glycosyl linking group, which is interposed between, and covalently linked to, both the peptide (directly or through an intervening glycosyl linker) and the modifying group (*e.g.*, water-  
10 soluble polymer). The glycosyl linking group includes a modified saccharyl fragment. The method includes contacting the glycopeptide with an activated modified saccharyl fragment and an enzyme for which the activated modified saccharyl fragment is a substrate. The components of the reaction mixture are combined under conditions appropriate to enzymatically transfer the modified saccharyl fragment from the activated modified saccharyl  
15 fragment to an acceptor moiety on the glycopeptide, thereby preparing the conjugate.

[0252] The acceptor peptide is typically synthesized *de novo*, or recombinantly expressed in a prokaryotic cell (*e.g.*, bacterial cell, such as *E. coli*) or in a eukaryotic cell such as a mammalian, yeast, insect, fungal or plant cell. The peptide can be either a full-length protein or a fragment. Moreover, the peptide can be a wild type or mutated peptide. In an exemplary  
20 embodiment, the peptide includes a mutation that adds one or more N- or O-linked glycosylation sites to the peptide sequence.

[0253] The method of the invention also provides for modification of incompletely glycosylated peptides that are produced recombinantly. Many recombinantly produced glycoproteins are incompletely glycosylated, exposing carbohydrate residues that may have undesirable properties, *e.g.*, immunogenicity, recognition by the RES. The incomplete  
25 glycosyl residue can be masked using a water-soluble polymer.

[0254] Exemplary peptides that can be modified using the methods of the invention are set forth in FIG.1.

[0255] Peptides modified by the methods of the invention can be synthetic or wild-type  
30 peptides or they can be mutated peptides, produced by methods known in the art, such as site-directed mutagenesis. Glycosylation of peptides is typically either N-linked or O-linked. An

exemplary N-linkage is the attachment of the modified saccharyl fragment to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of a carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one sugar (*e.g.*, N-acetylgalactosamine, galactose, mannose, GlcNAc, glucose, fucose or xylose) to the hydroxy side chain of a hydroxyamino acid, preferably serine or threonine, although unusual or non-natural amino acids, *e.g.*, 5-hydroxyproline or 5-hydroxylysine may also be used.

[0256] Moreover, in addition to peptides, the methods of the present invention can be practiced with other biological structures (*e.g.*, glycolipids, lipids, sphingoids, ceramides, whole cells, and the like. In general, the only limitation on the substrate structure is that it includes a glycosylation site).

[0257] For substrates lacking a glycosylation site, or for which it is desired to add a further glycosylation site, reliable methods are known in the art. For example, addition of glycosylation sites to a peptide, or other structure, is conveniently accomplished by altering the amino acid sequence such that it contains the desired glycosylation site. The addition may be made by mutation or by full chemical synthesis of the peptide. The peptide amino acid sequence is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the peptide at preselected bases such that codons are generated that will translate into the desired amino acids. The DNA mutation(s) are preferably made using methods known in the art. Both O-linked and N-linked glycosylation sites can be engineered into a peptide.

[0258] In an exemplary embodiment, the glycosylation site is added by shuffling polynucleotides. Polynucleotides encoding a candidate peptide can be modulated with DNA shuffling protocols. DNA shuffling is a process of recursive recombination and mutation, performed by random fragmentation of a pool of related genes, followed by reassembly of the fragments by a polymerase chain reaction-like process. *See, e.g.*, Stemmer, *Proc. Natl. Acad. Sci. USA* **91**: 10747-10751 (1994); Stemmer, *Nature* **370**: 389-391 (1994); and U.S. Patent Nos. 5,605,793, 5,837,458, 5,830,721 and 5,811,238.

[0259] The present invention also provides means of adding (or removing) one or more selected glycosyl residues to a peptide, after which a modified saccharyl fragment is

conjugated to at least one of the selected glycosyl residues of the peptide. The present embodiment is useful, for example, when it is desired to conjugate the modified saccharyl fragment to a selected glycosyl residue that is either not present on a peptide or is not present in a desired amount. Thus, prior to coupling a modified saccharyl fragment to a peptide, the 5 selected glycosyl residue is conjugated to the peptide by enzymatic or chemical coupling. In another embodiment, the glycosylation pattern of a glycopeptide is altered prior to the conjugation of the modified saccharyl fragment by the removal of a carbohydrate residue from the glycopeptide. See, for example WO 98/31826.

[0260] Addition or removal of any carbohydrate moiety present on the glycopeptide is 10 accomplished either chemically or enzymatically. Chemical deglycosylation is preferably brought about by exposure of the polypeptide variant to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the peptide intact. Chemical deglycosylation is 15 described by Hakimuddin *et al.*, *Arch. Biochem. Biophys.* **259**: 52 (1987) and by Edge *et al.*, *Anal. Biochem.* **118**: 131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptide variants can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.*, *Meth. Enzymol.* **138**: 350 (1987).

[0261] Chemical addition of glycosyl moieties is carried out by any art-recognized method. 20 Enzymatic addition of sugar moieties is preferably achieved using a modification of the methods set forth herein, substituting native glycosyl units for the modified saccharyl fragments used in the invention. Other methods of adding sugar moieties are disclosed in U.S. Patent No. 5,876,980, 6,030,815, 5,728,554, and 5,922,577.

[0262] Exemplary attachment points for selected glycosyl residue include, but are not 25 limited to: (a) consensus sites for N-linked glycosylation, and sites for O-linked glycosylation; (b) terminal glycosyl moieties that are acceptors for a glycosyltransferase; (c) arginine, asparagine and histidine; (d) free carboxyl groups; (e) free sulfhydryl groups such as those of cysteine; (f) free hydroxyl groups such as those of serine, threonine, or hydroxyproline; (g) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan; 30 or (h) the amide group of glutamine. Exemplary methods of use in the present invention are described in WO 87/05330 published Sep. 11, 1987, and in Aplin and Wriston, CRC CRIT. REV. BIOCHEM., pp. 259-306 (1981).

[0263] In one embodiment, the invention provides a method for linking two or more peptides through a linking group. The linking group is of any useful structure and may be selected from straight- and branched-chain structures. Preferably, each terminus of the linker, which is attached to a peptide, includes a modified saccharyl fragment.

5 [0264] In an exemplary method of the invention, two peptides are linked together via a linker moiety that includes a polymeric (e.g., PEG linker). The focus on a PEG linker that includes two glycosyl groups is for purposes of clarity and should not be interpreted as limiting the identity of linker arms of use in this embodiment of the invention. In an example of this embodiment, diamino-PEG is converted to a bifunctional linking group by reaction  
10 with two saccharyl fragments, e.g., sialic acid aldehyde. The bifunctional linking group is then enzymatically coupled to each peptide. As will be appreciated by those of skill in the art, the saccharyl fragments attached to the PEG moiety can be the same or different.

15 [0265] Exemplary peptides with which the present invention can be practiced, methods of adding or removing glycosylation sites, and adding or removing glycosyl structures or substructures are described in detail in WO03/031464 and related U.S. and PCT applications.

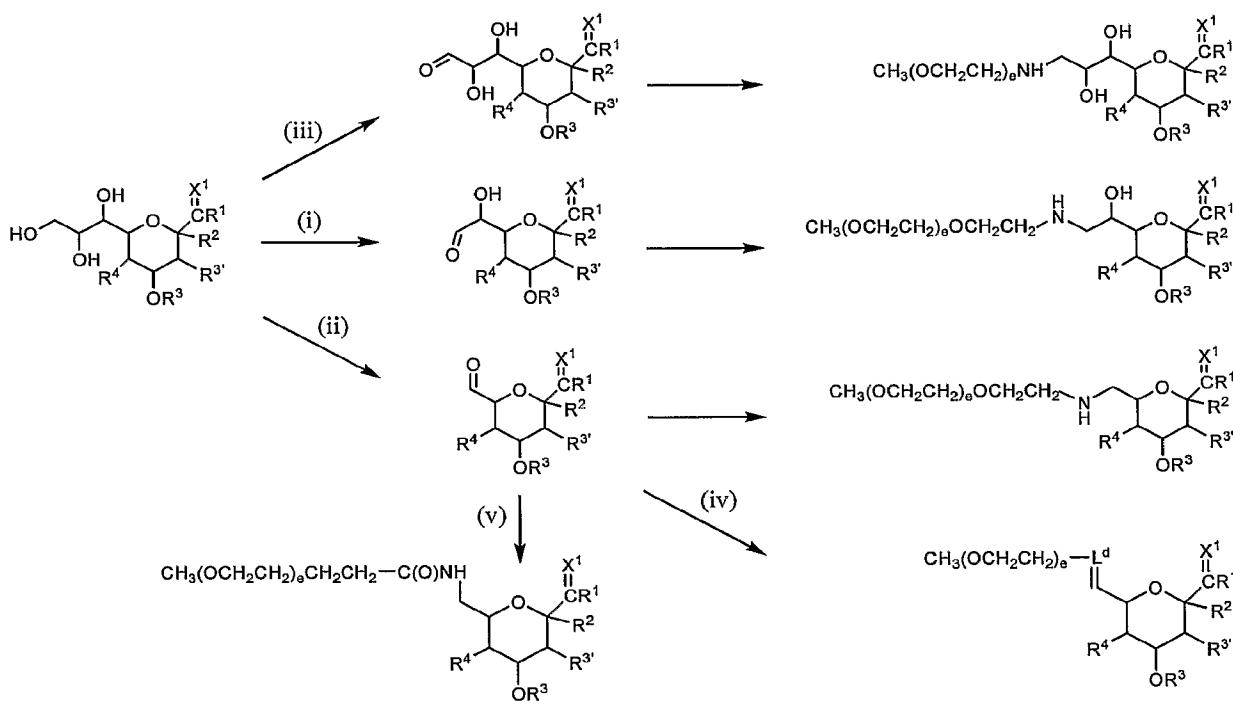
#### Preparation of Modified Saccharyl Fragments

20 [0266] In general, the saccharyl fragment and the modifying group are linked together through the use of reactive groups, which are typically transformed by the linking process into a new organic functional group or unreactive species. The reactive group on the saccharyl fragment is generally formed through a degradative process, e.g., oxidation. In the present invention, the modified saccharyl fragment is generally made by combining an amino analogue of the modifying group with an aldehyde or ketone moiety generated by oxidation of a saccharyl hydroxyl moiety.

25 [0267] In an exemplary embodiment, the method provides for forming a covalent conjugate between a modified saccharyl fragment and a glycosylated or non-glycosylated peptide. The method includes enzymatically transferring the modified saccharyl fragment from an activated modified saccharyl fragment to an acceptor moiety on the peptide. In another exemplary embodiment, the modified saccharyl fragment is covalently attached to a glycosyl residue that is covalently attached to the peptide. In another exemplary embodiment, the modified saccharyl fragment is covalently attached to an amino acid residue of the peptide.  
30 In another exemplary embodiment, the enzyme is a glycosyltransferase which is a member

selected from sialyltransferases, trans-sialidases, galactosyltransferases, glucosyltransferases, GalNAc transferase, GlcNAc transferase, fucosyltransferases, and mannosyltransferases. In another exemplary embodiment, the glycosyltransferase is recombinant. In another exemplary embodiment, the method is performed in a cell-free environment.

5 [0268] Methods for converting saccharyl hydroxyl moieties into carbonyl-containing compounds are well known in the art. As exemplified by the selective oxidation of the side chain of sialic acid, conditions are generally available for preparing an oxidized saccharyl precursor in a controlled and reproducible fashion.



10 [0269] For example, in the scheme above, selective oxidation of the primary hydroxyl of the sialic acid side chain, followed by reductive amination with m-PEG-NH<sub>2</sub> provides the corresponding saccharyl PEG-amine fragment according to route (iii).

[0270] Further, mild periodate oxidation (e.g., 1 mM sodium metaperiodate, 0 °C), according to route (i), produces a sialic acid fragment that is incompletely oxidized relative to 15 the fragment resulting from the harsher oxidation conditions of route (ii). The aldehyde is coupled with a modifying group, e.g., amino-m-PEG, under reducing conditions, thereby forming an exemplary sialic acid fragment-m-PEG conjugate.

[0271] As shown in route (iv), the oxidized sialic acid can also be reacted with a Wittig, Grignard or lithium reagent to form a species in which the water-soluble polymer and the saccharyl fragment are conjugated through a linker group, L<sup>d</sup>. The alkene moiety can be reduced using art-recognized conditions, forming a species in which L<sup>d</sup> is linked to the remainder of the saccharyl fragment through a saturated C-C bond. Exemplary linkers include substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl moieties.

[0272] Route (v) exemplifies a scheme in which the aldhehyde is reductively aminated with ammonia and the resulting amine is acylated with an active m-PEG derivative, e.g., an active ester.

[0273] Those of skill in the art will readily appreciate that both routes (iv) and (v) can be practiced with any of the side chain oxidized sialic acid fragments set forth in the scheme.

[0274] In addition to the species described above, R<sup>1</sup>-R<sup>4</sup> can also represent or include protecting groups or protected groups. Those of skill in the art understand how to protect a particular functional group such that it does not interfere with a chosen set of reaction conditions. For examples of useful protecting groups, see, for example, Greene *et al.*, PROTECTIVE GROUPS IN ORGANIC SYNTHESIS, John Wiley & Sons, New York, 1991.

[0275] Although exemplified above by reference to the use of an amine analogue of the modifying group, it is understood that the aldehyde or ketone group of the saccharide is readily modified by via formation of carbonyl derivatives such as, for example, imines, hydrazones, semicarbazones or oximes, or via such mechanisms as Grignard addition or alkylolithium addition. Accordingly, the present invention encompasses modified saccharyl fragments, linking groups and conjugates that include one or more of these derivatives, and is not limited to a particular saccharyl fragment or method of forming the fragment.

[0276] Exemplary moieties attached to the conjugates disclosed herein include, but are not limited to, PEG derivatives (e.g., acyl-PEG, acyl-alkyl-PEG, alkyl-acyl-PEG carbamoyl-PEG, aryl-PEG), PPG derivatives (e.g., acyl-PPG, acyl-alkyl-PPG, alkyl-acyl-PPG carbamoyl-PPG, aryl-PPG), therapeutic moieties, diagnostic moieties, mannose-6-phosphate, heparin, heparan, SLe<sub>x</sub>, mannose, mannose-6-phosphate, Sialyl Lewis X, FGF, VFGF, proteins, chondroitin, keratan, dermatan, albumin, integrins, antennary oligosaccharides, peptides and the like. Methods of conjugating the various modifying groups to a saccharide

moiety are readily accessible to those of skill in the art (POLY(ETHYLENE GLYCOL) CHEMISTRY: BIOTECHNICAL AND BIOMEDICAL APPLICATIONS, J. Milton Harris, Ed., Plenum Pub. Corp., 1992; POLY(ETHYLENE GLYCOL) CHEMICAL AND BIOLOGICAL APPLICATIONS, J. Milton Harris, Ed., ACS Symposium Series No. 680, American Chemical Society, 1997;

5 Hermanson, BIOCONJUGATE TECHNIQUES, Academic Press, San Diego, 1996; and Dunn *et al.*,  
Eds. POLYMERIC DRUGS AND DRUG DELIVERY SYSTEMS, ACS Symposium Series Vol. 469,  
American Chemical Society, Washington, D.C. 1991).

## Cross-linking Groups

[0277] Preparation of the modified saccharyl fragment for use in the methods of the present invention includes attachment of a modifying group to a sugar residue and forming a stable adduct, which is a substrate for a glycosyltransferase. Thus, it is often preferred to use a cross-linking agent to conjugate the modifying group and the sugar. Exemplary bifunctional compounds which can be used for attaching modifying groups to carbohydrate moieties include, but are not limited to, bifunctional poly(ethyleneglycols), polyamides, polyethers, polyesters and the like. General approaches for linking carbohydrates to other molecules are known in the literature. See, for example, Lee *et al.*, *Biochemistry* **28**: 1856 (1989); Bhatia *et al.*, *Anal. Biochem.* **178**: 408 (1989); Janda *et al.*, *J. Am. Chem. Soc.* **112**: 8886 (1990) and Bednarski *et al.*, WO 92/18135. In the discussion that follows, the reactive groups are treated as benign on the sugar moiety of the nascent modified saccharyl fragment. The focus of the discussion is for clarity of illustration. Those of skill in the art will appreciate that the discussion is relevant to reactive groups on the modifying group as well.

[0278] A variety of reagents are used to modify the components of the modified saccharyl fragment with intramolecular chemical crosslinks (for reviews of crosslinking reagents and crosslinking procedures see: Wold, F., *Meth. Enzymol.* **25**: 623-651, 1972; Weetall, H. H., and Cooney, D. A., In: ENZYMES AS DRUGS. (Holcenberg, and Roberts, eds.) pp. 395-442, Wiley, New York, 1981; Ji, T. H., *Meth. Enzymol.* **91**: 580-609, 1983; Mattson *et al.*, *Mol. Biol. Rep.* **17**: 167-183, 1993, all of which are incorporated herein by reference). Preferred crosslinking reagents are derived from various zero-length, homo-bifunctional, and hetero-bifunctional crosslinking reagents. Zero-length crosslinking reagents include direct conjugation of two intrinsic chemical groups with no introduction of extrinsic material. Agents that catalyze formation of a disulfide bond belong to this category. Another example is reagents that induce condensation of a carboxyl and a primary amino group to form an

amide bond such as carbodiimides, ethylchloroformate, Woodward's reagent K (2-ethyl-5-phenylisoxazolium-3'-sulfonate), and carbonyldiimidazole. In addition to these chemical reagents, the enzyme transglutaminase (glutamyl-peptide  $\gamma$ -glutamyltransferase; EC 2.3.2.13) may be used as zero-length crosslinking reagent. This enzyme catalyzes acyl transfer  
5 reactions at carboxamide groups of protein-bound glutamyl residues, usually with a primary amino group as substrate. Preferred homo- and hetero-bifunctional reagents contain two identical or two dissimilar sites, respectively, which may be reactive for amino, sulphydryl, guanidino, indole, or nonspecific groups.

[0279] An exemplary cross-linking moiety includes a reactive functional group that reacts  
10 with the saccharyl ketone or aldehyde moiety (e.g., amine, hydrazine, etc.). The reactive functional group is tethered to a second reactive functional group that reacts with a moiety on the modifying group, forming a linker covalently bonded to both the saccharyl fragment and the modifying group.

[0280] Exemplary cross-linking groups of use in the present invention are set forth in  
15 WO03/031464 and related U.S. and PCT applications.

#### Conjugation of Modified Saccharyl Fragments to Peptides

[0281] The modified saccharyl fragments are conjugated to a glycosylated or non-glycosylated peptide using an appropriate enzyme to mediate the conjugation. Preferably, the concentrations of the modified donor sugar(s), enzyme(s) and acceptor peptide(s) are selected  
20 such that glycosylation proceeds until the acceptor is consumed. The considerations discussed below, while set forth in the context of a sialyltransferase, are generally applicable to other glycosyltransferase reactions.

[0282] A number of methods of using glycosyltransferases to synthesize desired oligosaccharide structures are known and are generally applicable to the instant invention.  
25 Exemplary methods are described, for instance, WO 96/32491, Ito *et al.*, *Pure Appl. Chem.*  
65: 753 (1993), and U.S. Pat. Nos. 5,352,670, 5,374,541, and 5,545,553.

[0283] The present invention is practiced using a single glycosyltransferase or a combination of glycosyltransferases. For example, one can use a combination of a sialyltransferase and a galactosyltransferase. In those embodiments using more than one  
30 enzyme, the enzymes and substrates are preferably combined in an initial reaction mixture, or the enzymes and reagents for a second enzymatic reaction are added to the reaction medium

once the first enzymatic reaction is complete or nearly complete. By conducting two enzymatic reactions in sequence in a single vessel, overall yields are improved over procedures in which an intermediate species is isolated. Moreover, cleanup and disposal of extra solvents and by-products is reduced.

5 [0284] In a preferred embodiment, each of the first and second enzyme is a glycosyltransferase. In another preferred embodiment, one enzyme is an endoglycosidase. In an additional preferred embodiment, more than two enzymes are used to assemble the modified glycoprotein of the invention. The enzymes are used to alter a saccharide structure on the peptide at any point either before or after the addition of the modified saccharyl fragment to the peptide.

10 [0285] In another preferred embodiment, each of the enzymes utilized to produce a conjugate of the invention are present in a catalytic amount. The catalytic amount of a particular enzyme varies according to the concentration of that enzyme's substrate as well as to reaction conditions such as temperature, time and pH value. Means for determining the 15 catalytic amount for a given enzyme under preselected substrate concentrations and reaction conditions are well known to those of skill in the art.

[0286] The temperature at which an above process is carried out can range from just above freezing to the temperature at which the most sensitive enzyme denatures. Preferred temperature ranges are about 0 °C to about 45 °C, and more preferably about 20 °C to about 20 30 °C. In another exemplary embodiment, one or more components of the present method are conducted at an elevated temperature using a thermophilic enzyme.

25 [0287] The reaction mixture is maintained for a period of time sufficient for the acceptor to be glycosylated, thereby forming the desired conjugate. Some of the conjugate can often be detected after a few hours, with recoverable amounts usually being obtained within 24 hours or less. Those of skill in the art understand that the rate of reaction is dependent on a number of variable factors (*e.g.*, enzyme concentration, donor concentration, acceptor concentration, temperature, solvent volume), which are optimized for a selected system.

[0288] The present invention also provides for the industrial-scale production of modified peptides.

30 [0289] In the discussion that follows, the invention is exemplified by the conjugation of modified sialic acid fragment to a glycosylated peptide. The exemplary modified sialic acid

fragment is labeled with PEG. The focus of the following discussion on the use of PEG-modified sialic acid fragments and glycosylated peptides is for clarity of illustration and is not intended to imply that the invention is limited to the conjugation of these two partners. One of skill understands that the discussion is generally applicable to the additions of 5 modified glycosyl fragments other than sialic acid fragments. Moreover, the discussion is equally applicable to the modification of a saccharyl fragment with agents other than PEG including other water-soluble polymers, therapeutic moieties, and biomolecules.

[0290] An enzymatic approach can be used for the selective introduction of PEGylated or PPGylated carbohydrates onto a peptide or glycopeptide. The method utilizes modified 10 saccharyl fragments containing PEG, PPG, or a masked reactive functional group, and is combined with the appropriate glycosyltransferase or glycosynthase. By selecting the glycosyltransferase that will make the desired carbohydrate linkage and utilizing the modified saccharyl fragment as the donor substrate, the PEG or PPG can be introduced directly onto the peptide backbone, onto existing sugar residues of a glycopeptide or onto sugar residues 15 that have been added to a peptide.

[0291] An acceptor for the sialyltransferase is present on the peptide to be modified by the methods of the present invention either as a naturally occurring structure or one placed there recombinantly, enzymatically or chemically. Suitable acceptors, include, for example, galactosyl acceptors such as GalNAc, Gal $\beta$ 1,4GlcNAc, Gal $\beta$ 1,4GalNAc, Gal $\beta$ 1,3GalNAc, 20 lacto-N-tetraose, Gal $\beta$ 1,3GlcNAc, Gal $\beta$ 1,3Ara, Gal $\beta$ 1,6GlcNAc, Gal $\beta$ 1,4Glc (lactose), and other acceptors known to those of skill in the art (*see, e.g.*, Paulson *et al.*, *J. Biol. Chem.* **253**: 5617-5624 (1978)).

[0292] In one embodiment, an acceptor for the sialyltransferase is present on the glycopeptide to be modified upon *in vivo* synthesis of the glycopeptide. Such glycopeptides 25 can be sialylated using the claimed methods without prior modification of the glycosylation pattern of the glycopeptide. Alternatively, the methods of the invention can be used to sialylate a peptide that does not include a suitable acceptor; one first modifies the peptide to include an acceptor by methods known to those of skill in the art. In an exemplary embodiment, a GalNAc residue is added by the action of a GalNAc transferase.

[0293] In an exemplary embodiment, an acceptor for a modified sialic acid fragment is assembled by attaching a galactose residue to an appropriate acceptor linked to the peptide, e.g., a GlcNAc. The method includes incubating the peptide to be modified with a reaction

mixture that contains a suitable amount of a galactosyltransferase (*e.g.*, gal $\beta$ 1,3 or gal $\beta$ 1,4), and a suitable galactosyl donor (*e.g.*, UDP-galactose). The reaction is allowed to proceed substantially to completion or, alternatively, the reaction is terminated when a preselected amount of the galactose residue is added. Other methods of assembling a selected saccharide acceptor will be apparent to those of skill in the art.

[0294] In yet another embodiment, glycopeptide-linked oligosaccharides are first “trimmed,” either in whole or in part, to expose either an acceptor for the sialyltransferase or a moiety to which one or more appropriate residues can be added to obtain a suitable acceptor. Enzymes such as glycosyltransferases and endoglycosidases (*see*, for example U.S. Patent No. 5,716,812) are useful for the attaching and trimming reactions.

[0295] In the discussion that follows, the method of the invention is exemplified by the use of modified saccharyl fragments having a water-soluble polymer attached thereto. The focus of the discussion is for clarity of illustration. Those of skill will appreciate that the discussion is equally relevant to those embodiments in which the modified saccharyl fragment bears a therapeutic moiety, biomolecule or the like.

[0296] In another exemplary embodiment, a water-soluble polymer is added to one or both of the terminal mannose residues of the biantennary structure via a modified saccharyl fragment having a galactose residue, which is conjugated to a GlcNAc residue added onto the terminal mannose residues. Alternatively, an unmodified Gal can be added to one or both terminal GlcNAc residues.

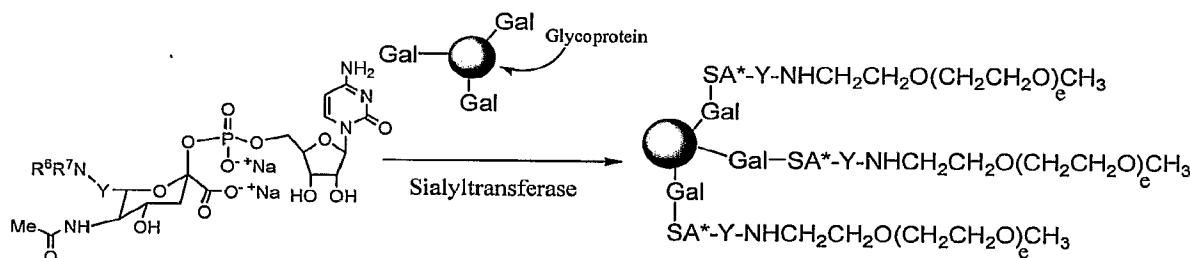
[0297] In yet a further example, a water-soluble polymer is added onto a Gal residue using a modified sialic acid fragment.

[0298] The Examples set forth above provide an illustration of the power of the methods set forth herein. Using the methods of the invention, it is possible to “trim back” and build up a carbohydrate residue of substantially any desired structure. The modified saccharyl fragment can be added to the termini of the carbohydrate moiety as set forth above, or it can be intermediate between the peptide core and the terminus of the carbohydrate.

[0299] In an exemplary embodiment, an existing sialic acid is removed from a glycopeptide using a sialidase, thereby unmasking all or most of the underlying galactosyl residues. Alternatively, a peptide or glycopeptide is labeled with galactose residues, or an oligosaccharide residue that terminates in a galactose unit. Following the exposure of, or

addition of, the galactose residues, an appropriate sialyltransferase is used to add a modified sialic acid. The approach is summarized in Scheme 2.

**Scheme 2**



5 In which SA\* is saccharyl fragment and Y is as described above (Formula I).

[0300] In an alternative embodiment, the modified saccharyl fragment is added directly to the peptide backbone using a glycosyltransferase known to transfer sugar residues to the peptide backbone. Use of this approach allows the direct addition of modified saccharyl fragments onto peptides that lack any carbohydrates or, alternatively, onto existing

10 glycopeptides. In both cases, the addition of the modified saccharyl fragment occurs at specific positions on the peptide backbone as defined by the substrate specificity of the glycosyltransferase and not in a random manner as occurs during modification of a protein's peptide backbone using chemical methods. An array of agents can be introduced into proteins or glycopeptides that lack the glycosyltransferase substrate peptide sequence by 15 engineering the appropriate amino acid sequence into the polypeptide chain.

[0301] In each of the exemplary embodiments set forth above, one or more additional chemical or enzymatic modification steps can be utilized following the conjugation of the modified saccharyl fragment to the peptide. In an exemplary embodiment, an enzyme (*e.g.*, fucosyltransferase) is used to append a glycosyl unit (*e.g.*, fucose) onto the terminal modified 20 saccharyl fragment attached to the peptide. In another example, an enzymatic reaction is utilized to “cap” sites to which the modified saccharyl fragment failed to conjugate. Alternatively, a chemical reaction is utilized to alter the structure of the conjugated modified saccharyl fragment. For example, the conjugated modified saccharyl fragment is reacted with agents that stabilize or destabilize its linkage with the peptide component to which the 25 modified saccharyl fragment is attached. In another example, a component of the modified saccharyl fragment is deprotected following its conjugation to the peptide. One of skill will appreciate that there is an array of enzymatic and chemical procedures that are useful in the

methods of the invention at a stage after the modified saccharyl fragment is conjugated to the peptide. Further elaboration of the modified saccharyl fragment-peptide conjugate is within the scope of the invention.

[0302] In another exemplary embodiment, the invention provides a composition for forming a conjugate between a peptide and a modified saccharyl fragment. This composition includes a mixture of an activated modified saccharyl fragment, an enzyme for which the activated modified saccharyl fragment is a substrate, and a peptide acceptor substrate, wherein the modified saccharyl fragment is covalently attached a member selected from water-soluble polymers, therapeutic moieties and biomolecules.

10 **Enzymes**

[0303] General methods of remodeling peptides and lipids using enzymes that transfer a sugar donor to an acceptor are discussed in detail in DeFrees, WO 03/031464 A2, published April 17, 2003. A brief summary of selected enzymes of use in the present method is set forth below.

15 **Glycosyltransferases**

[0304] Glycosyltransferases catalyze the addition of activated sugars (donor NDP-sugars), in a step-wise fashion, to a protein, glycopeptide, lipid or glycolipid or to the non-reducing end of a growing oligosaccharide. N-linked glycopeptides are synthesized via a transferase and a lipid-linked oligosaccharide donor Dol-PP-NAG<sub>2</sub>Glc<sub>3</sub>Man<sub>9</sub> in an en block transfer followed by trimming of the core. In this case the nature of the "core" saccharide is somewhat different from subsequent attachments. A very large number of glycosyltransferases are known in the art.

[0305] The glycosyltransferase to be used in the present invention may be any as long as it can utilize the modified saccharyl fragment as a sugar donor. Examples of such enzymes include Leloir pathway glycosyltransferase, such as galactosyltransferase, N-acetylglucosaminyltransferase, N-acetylgalactosaminyltransferase, fucosyltransferase, sialyltransferase, mannosyltransferase, xylosyltransferase, glucurononyltransferase and the like.

[0306] For enzymatic saccharide syntheses that involve glycosyltransferase reactions, glycosyltransferase can be cloned, or isolated from any source. Many cloned glycosyltransferases are known, as are their polynucleotide sequences. See, e.g., "The WWW

Guide To Cloned Glycosyltransferases," Taniguchi et al., 2002, Handbook of Glycosyltransferases and Related Genes, Springer, Tokyo. Glycosyltransferase amino acid sequences and nucleotide sequences encoding glycosyltransferases from which the amino acid sequences can be deduced are also found in various publicly available databases, 5 including GenBank, Swiss-Prot, EMBL, and others.

[0307] Glycosyltransferases that can be employed in the methods of the invention include, but are not limited to, galactosyltransferases, fucosyltransferases, glucosyltransferases, N-acetylgalactosaminyltransferases, N-acetylglicosaminyltransferases, glucuronyltransferases, sialyltransferases, mannosyltransferases, glucuronic acid transferases, galacturonic acid 10 transferases, and oligosaccharyltransferases. Suitable glycosyltransferases include those obtained from eukaryotes, as well as from prokaryotes. The enzymes may be wild-type or mutant enzymes. Methods of preparing mutant glycosyltransferases and characterizing these species are known in the art.

#### Fucosyltransferases

[0308] In some embodiments, a glycosyltransferase used in the method of the invention is a fucosyltransferase. Fucosyltransferases are known to those of skill in the art. Exemplary fucosyltransferases include enzymes, which transfer L-fucose from GDP-fucose to a hydroxy position of an acceptor sugar. Fucosyltransferases that transfer non-nucleotide sugars to an acceptor are also of use in the present invention.

[0309] In some embodiments, the acceptor sugar is, for example, the GlcNAc in a Gal $\beta$ (1 $\rightarrow$ 3,4)GlcNAc $\beta$ - group in an oligosaccharide glycoside. Suitable fucosyltransferases for this reaction include the Gal $\beta$ (1 $\rightarrow$ 3,4)GlcNAc $\beta$ 1- $\alpha$ (1 $\rightarrow$ 3,4)fucosyltransferase (FTIII E.C. No. 2.4.1.65), which was first characterized from human milk (see, Palcic, et al., *Carbohydrate Res.* **190**: 1-11 (1989); Prieels, et al., *J. Biol. Chem.* **256**: 10456-10463 (1981); 20 and Nunez, et al., *Can. J. Chem.* **59**: 2086-2095 (1981)) and the Gal $\beta$ (1 $\rightarrow$ 4)GlcNAc $\beta$ - $\alpha$ fucosyltransferases (FTIV, FTV, FTVI) which are found in human serum. FTVII (E.C. No. 2.4.1.65), a sialyl  $\alpha$ (2 $\rightarrow$ 3)Gal $\beta$ ((1 $\rightarrow$ 3)GlcNAc $\beta$  fucosyltransferase, has also been characterized. A recombinant form of the Gal $\beta$ (1 $\rightarrow$ 3,4) GlcNAc $\beta$ - $\alpha$ (1 $\rightarrow$ 3,4)fucosyltransferase has also been characterized (see, Dumas, et al., *Bioorg. Med. Letters* **1**: 425-428 (1991) and Kukowska-Latallo, et al., *Genes and Development* **4**: 1288-30 1303 (1990)). Other exemplary fucosyltransferases include, for example,  $\alpha$ 1,2

fucosyltransferase (E.C. No. 2.4.1.69). Enzymatic fucosylation can be carried out by the methods described in Mollicone, *et al.*, *Eur. J. Biochem.* **191**: 169-176 (1990) or U.S. Patent No. 5,374,655. Cells that are used to produce a fucosyltransferase will also include an enzymatic system for synthesizing GDP-fucose.

5     Galactosyltransferases

[0310] In another group of embodiments, the glycosyltransferase is a galactosyltransferase. Exemplary galactosyltransferases include  $\alpha$ (1,3) galactosyltransferases (E.C. No. 2.4.1.151, see, e.g., Dabkowski *et al.*, *Transplant Proc.* **25**:2921 (1993) and Yamamoto *et al.* *Nature* **345**: 229-233 (1990), bovine (GenBank j04989, Joziasse *et al.*, *J. Biol. Chem.* **264**: 14290-10 14297 (1989)), murine (GenBank m26925; Larsen *et al.*, *Proc. Nat'l. Acad. Sci. USA* **86**: 8227-8231 (1989)), porcine (GenBank L36152; Strahan *et al.*, *Immunogenetics* **41**: 101-105 (1995)). Another suitable  $\alpha$ 1,3 galactosyltransferase is that which is involved in synthesis of the blood group B antigen (EC 2.4.1.37, Yamamoto *et al.*, *J. Biol. Chem.* **265**: 1146-1151 (1990) (human)). Yet a further exemplary galactosyltransferase is core Gal-T1.

15    [0311] Also suitable for use in the methods of the invention are  $\beta$ (1,4) galactosyltransferases, which include, for example, EC 2.4.1.90 (LacNAc synthetase) and EC 2.4.1.22 (lactose synthetase) (bovine (D'Agostaro *et al.*, *Eur. J. Biochem.* **183**: 211-217 (1989)), human (Masri *et al.*, *Biochem. Biophys. Res. Commun.* **157**: 657-663 (1988)), murine (Nakazawa *et al.*, *J. Biochem.* **104**: 165-168 (1988)), as well as E.C. 2.4.1.38 and the 20 ceramide galactosyltransferase (EC 2.4.1.45, Stahl *et al.*, *J. Neurosci. Res.* **38**: 234-242 (1994)). Other suitable galactosyltransferases include, for example,  $\alpha$ 1,2 galactosyltransferases (from e.g., *Schizosaccharomyces pombe*, Chapell *et al.*, *Mol. Biol. Cell* **5**: 519-528 (1994)).

Sialyltransferases

25    [0312] Sialyltransferases are another type of glycosyltransferase that is useful in the recombinant cells and reaction mixtures of the invention. Cells that produce recombinant sialyltransferases will also produce CMP-sialic acid, which is a sialic acid donor for sialyltransferases. Examples of sialyltransferases that are suitable for use in the present invention include ST3Gal III (e.g., a rat or human ST3Gal III), ST3Gal IV, ST3Gal I, ST6Gal 30 I, ST3Gal V, ST6Gal II, ST6GalNAc I, ST6GalNAc II, and ST6GalNAc III (the sialyltransferase nomenclature used herein is as described in Tsuji *et al.*, *Glycobiology* **6**: v-

xiv (1996)). An exemplary  $\alpha$ (2,3)sialyltransferase referred to as  $\alpha$ (2,3)sialyltransferase (EC 2.4.99.6) transfers sialic acid to the non-reducing terminal Gal of a Gal $\beta$ 1 $\rightarrow$ 3Glc disaccharide or glycoside. See, Van den Eijnden *et al.*, *J. Biol. Chem.* **256**: 3159 (1981), Weinstein *et al.*, *J. Biol. Chem.* **257**: 13845 (1982) and Wen *et al.*, *J. Biol. Chem.* **267**: 21011 (1992). Another exemplary  $\alpha$ 2,3-sialyltransferase (EC 2.4.99.4) transfers sialic acid to the non-reducing terminal Gal of the disaccharide or glycoside. see, Rearick *et al.*, *J. Biol. Chem.* **254**: 4444 (1979) and Gillespie *et al.*, *J. Biol. Chem.* **267**: 21004 (1992). Further exemplary enzymes include Gal- $\beta$ -1,4-GlcNAc  $\alpha$ -2,6 sialyltransferase (See, Kurosawa *et al.* *Eur. J. Biochem.* **219**: 375-381 (1994)).

10 [0313] A list of sialyltransferases of use in the invention are provided in FIG.2.

GalNAc transferases

[0314] N-acetylgalactosaminyltransferases are of use in practicing the present invention, particularly for binding a GalNAc moiety to an amino acid of the O-linked glycosylation site of the peptide. Suitable N-acetylgalactosaminyltransferases include, but are not limited to,  $\alpha$ (1,3) N-acetylgalactosaminyltransferase,  $\beta$ (1,4) N-acetylgalactosaminyltransferases (Nagata *et al.*, *J. Biol. Chem.* **267**: 12082-12089 (1992) and Smith *et al.*, *J. Biol. Chem.* **269**: 15162 (1994)) and polypeptide N-acetylgalactosaminyltransferase (Homa *et al.*, *J. Biol. Chem.* **268**: 12609 (1993)). See also the work of W. Wakarchuk generally and U.S. Patent No. 6,723,545; and published U.S. Patent Application No. 2003/0180928; 2003/0157658; 2003/0157657; and 2003/0157656.

[0315] Production of proteins such as the enzyme GalNAc T<sub>I-XX</sub> from cloned genes by genetic engineering is well known. See, eg., U.S. Pat. No. 4,761,371. One method involves collection of sufficient samples, then the amino acid sequence of the enzyme is determined by N-terminal sequencing. This information is then used to isolate a cDNA clone encoding a full-length (membrane bound) transferase which upon expression in the insect cell line Sf9 resulted in the synthesis of a fully active enzyme. The acceptor specificity of the enzyme is then determined using a semiquantitative analysis of the amino acids surrounding known glycosylation sites in 16 different proteins followed by in vitro glycosylation studies of synthetic peptides. This work has demonstrated that certain amino acid residues are overrepresented in glycosylated peptide segments and that residues in specific positions

surrounding glycosylated serine and threonine residues may have a more marked influence on acceptor efficiency than other amino acid moieties.

Cell-Bound Glycosyltransferases

[0316] In another embodiment, the enzymes utilized in the method of the invention are cell-bound glycosyltransferases. Although many soluble glycosyltransferases are known (see, for example, U.S. Pat. No. 5,032,519), glycosyltransferases are generally in membrane-bound form when associated with cells. Many of the membrane-bound enzymes studied thus far are considered to be intrinsic proteins; that is, they are not released from the membranes by sonication and require detergents for solubilization. Surface glycosyltransferases have

been identified on the surfaces of vertebrate and invertebrate cells, and it has also been recognized that these surface transferases maintain catalytic activity under physiological conditions. However, the more recognized function of cell surface glycosyltransferases is for intercellular recognition (Roth, MOLECULAR APPROACHES to SUPRACELLULAR PHENOMENA, 1990).

[0317] Methods have been developed to alter the glycosyltransferases expressed by cells. For example, Larsen *et al.*, *Proc. Natl. Acad. Sci. USA* **86**: 8227-8231 (1989), report a genetic approach to isolate cloned cDNA sequences that determine expression of cell surface oligosaccharide structures and their cognate glycosyltransferases. A cDNA library generated from mRNA isolated from a murine cell line known to express UDP-galactose:β-D-galactosyl-1,4-N-acetyl-D-glucosaminide α-1,3-galactosyltransferase was transfected into COS-1 cells. The transfected cells were then cultured and assayed for α 1-3 galactosyltransferase activity.

[0318] Francisco *et al.*, *Proc. Natl. Acad. Sci. USA* **89**: 2713-2717 (1992), disclose a method of anchoring β-lactamase to the external surface of *Escherichia coli*. A tripartite fusion consisting of (i) a signal sequence of an outer membrane protein, (ii) a membrane-spanning section of an outer membrane protein, and (iii) a complete mature β-lactamase sequence is produced resulting in an active surface bound β-lactamase molecule. However, the Francisco method is limited only to prokaryotic cell systems and as recognized by the authors, requires the complete tripartite fusion for proper functioning.

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. . . . .

### Sulfotransferases

[0319] The invention also provides methods for producing peptides that include sulfated molecules, including, for example sulfated polysaccharides such as heparin, heparan sulfate, carragenen, and related compounds. Suitable sulfotransferases include, for example,  
5 chondroitin-6-sulphotransferase (chicken cDNA described by Fukuta *et al.*, *J. Biol. Chem.* **270**: 18575-18580 (1995); GenBank Accession No. D49915), glycosaminoglycan N-acetylglucosamine N-deacetylase/N-sulphotransferase 1 (Dixon *et al.*, *Genomics* **26**: 239-241 (1995); UL18918), and glycosaminoglycan N-acetylglucosamine N-deacetylase/N-sulphotransferase 2 (murine cDNA described in Orellana *et al.*, *J. Biol. Chem.* **269**: 2270-  
10 2276 (1994) and Eriksson *et al.*, *J. Biol. Chem.* **269**: 10438-10443 (1994); human cDNA described in GenBank Accession No. U2304).

### Glycosidases

[0320] This invention also encompasses the use of wild-type and mutant glycosidases. Mutant  $\beta$ -galactosidase enzymes have been demonstrated to catalyze the formation of  
15 disaccharides through the coupling of an  $\alpha$ -glycosyl fluoride to a galactosyl acceptor molecule. (Withers, U.S. Pat. No. 6,284,494; issued Sept. 4, 2001). Other glycosidases of use in this invention include, for example,  $\beta$ -glucosidases,  $\beta$ -galactosidases,  $\beta$ -mannosidases,  $\beta$ -acetyl glucosaminidases,  $\beta$ -N-acetyl galactosaminidases,  $\beta$ -xylosidases,  $\beta$ -fucosidases, cellulases, xylanases, galactanases, mannanases, hemicellulases, amylases, glucoamylases,  $\alpha$ -glucosidases,  $\alpha$ -galactosidases,  $\alpha$ -mannosidases,  $\alpha$ -N-acetyl glucosaminidases,  $\alpha$ -N-acetyl  
20 galactose-aminidases,  $\alpha$ -xylosidases,  $\alpha$ -fucosidases, and neuraminidases/sialidases.

### Immobilized Enzymes

[0321] The present invention also provides for the use of enzymes that are immobilized on a solid and/or soluble support. In an exemplary embodiment, there is provided a  
25 glycosyltransferase that is conjugated to a PEG via an intact glycosyl linker according to the methods of the invention. The PEG-linker-enzyme conjugate is optionally attached to solid support. The use of solid supported enzymes in the methods of the invention simplifies the work up of the reaction mixture and purification of the reaction product, and also enables the facile recovery of the enzyme. The glycosyltransferase conjugate is utilized in the methods  
30 of the invention. Other combinations of enzymes and supports will be apparent to those of skill in the art.

**Purification of Peptide Conjugates**

[0322] The products produced by the above processes can be used without purification.

However, it is usually preferred to recover the product. Standard, well-known techniques for recovery of modified peptides such as thin or thick layer chromatography, column

5 chromatography, ion exchange chromatography, or membrane filtration can be used. It is preferred to use membrane filtration, more preferably utilizing a reverse osmotic membrane, or one or more column chromatographic techniques for the recovery as is discussed hereinafter and in the literature cited herein. For instance, membrane filtration wherein the membranes have molecular weight cutoff of about 3000 to about 10,000 can be used to  
10 remove proteins such as glycosyl transferases. Nanofiltration or reverse osmosis can then be used to remove salts and/or purify the conjugates (*see, e.g.*, WO 98/15581). Nanofilter membranes are a class of reverse osmosis membranes that pass monovalent salts but retain polyvalent salts and uncharged solutes larger than about 100 to about 2,000 Daltons, depending upon the membrane used. Thus, in a typical application, conjugates prepared by  
15 the methods of the present invention will be retained in the membrane and contaminating salts will pass through.

[0323] If the modified glycoprotein is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration; optionally, the protein may be concentrated with a  
20 commercially available protein concentration filter, followed by separating the polypeptide variant from other impurities by one or more steps selected from immunoaffinity chromatography, ion-exchange column fractionation (*e.g.*, on diethylaminoethyl (DEAE) or matrices containing carboxymethyl or sulfopropyl groups), chromatography on Blue-Sepharose, CM Blue-Sepharose, MONO-Q, MONO-S, lentil lectin-Sepharose, WGA-  
25 Sepharose, Con A-Sepharose, Ether Toyopearl, Butyl Toyopearl, Phenyl Toyopearl, or protein A Sepharose, SDS-PAGE chromatography, silica chromatography, chromatofocusing, reverse phase HPLC (*e.g.*, silica gel with appended aliphatic groups), gel filtration using, *e.g.*, Sephadex molecular sieve or size-exclusion chromatography, chromatography on columns that selectively bind the polypeptide, and ethanol or ammonium  
30 sulfate precipitation.

[0324] Modified glycopeptides produced in culture are usually isolated by initial extraction from cells, enzymes, etc., followed by one or more concentration, salting-out, aqueous ion-

exchange, or size-exclusion chromatography steps. Additionally, the modified glycoprotein may be purified by affinity chromatography. Finally, HPLC may be employed for final purification steps.

[0325] A protease inhibitor, *e.g.*, methylsulfonylfluoride (PMSF) may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[0326] In another method, supernatants from systems that produce the modified glycopeptide of the invention are first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit.

10 Following the concentration step, the concentrate may be applied to a suitable purification matrix. For example, a suitable affinity matrix may comprise a ligand for the peptide, a lectin or antibody molecule bound to a suitable support. Alternatively, an anion-exchange resin may be employed, for example, a matrix or substrate having pendant DEAE groups. Suitable matrices include acrylamide, agarose, dextran, cellulose, or other types commonly employed in protein purification. Alternatively, a cation-exchange step may be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are particularly preferred.

15 [0327] Finally, one or more RP-HPLC steps employing hydrophobic RP-HPLC media, *e.g.*, silica gel having pendant methyl or other aliphatic groups, may be employed to further purify a polypeptide variant composition. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous modified glycoprotein.

20 [0328] The modified glycopeptide of the invention resulting from a large-scale fermentation may be purified by methods analogous to those disclosed by Urdal *et al.*, *J. Chromatog.* **296**: 171 (1984). This reference describes two sequential, RP-HPLC steps for 25 purification of recombinant human IL-2 on a preparative HPLC column. Alternatively, techniques such as affinity chromatography may be utilized to purify the modified glycoprotein.

### **Pharmaceutical Compositions**

[0329] In another aspect, the invention provides a pharmaceutical composition. The 30 pharmaceutical composition includes a pharmaceutically acceptable carrier and a conjugate between a glycosylated or non-glycosylated peptide and a modified saccharyl fragment which

is covalently linked to a water-soluble or -insoluble polymer, therapeutic moiety or biomolecule. The polymer, therapeutic moiety or biomolecule is conjugated to the peptide via an intact glycosyl linking group interposed between and covalently linked to both the peptide and the polymer, therapeutic moiety or biomolecule.

5 [0330] Pharmaceutical compositions of the invention are suitable for use in a variety of drug delivery systems. Suitable formulations for use in the present invention are found in *Remington's Pharmaceutical Sciences*, Mace Publishing Company, Philadelphia, PA, 17th ed. (1985). For a brief review of methods for drug delivery, see, Langer, *Science* 249:1527-1533 (1990).

10 [0331] The pharmaceutical compositions may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as  
15 mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

20 [0332] Commonly, the pharmaceutical compositions are administered parenterally, e.g., intravenously. Thus, the invention provides compositions for parenteral administration which comprise the compound dissolved or suspended in an acceptable carrier, preferably an aqueous carrier, e.g., water, buffered water, saline, PBS and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate  
25 physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents, detergents and the like.

[0333] These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to  
30 administration. The pH of the preparations typically will be between 3 and 11, more preferably from 5 to 9 and most preferably from 7 and 8.

[0334] In some embodiments the glycopeptides of the invention can be incorporated into liposomes formed from standard vesicle-forming lipids. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka *et al.*, *Ann. Rev. Biophys. Bioeng.* 9: 467 (1980), U.S. Pat. Nos. 4,235,871, 4,501,728 and 4,837,028. The targeting of liposomes using 5 a variety of targeting agents (e.g., the sialyl galactosides of the invention) is well known in the art (see, e.g., U.S. Patent Nos. 4,957,773 and 4,603,044).

[0335] Standard methods for coupling targeting agents to liposomes can be used. These methods generally involve incorporation into liposomes of lipid components, such as phosphatidylethanolamine, which can be activated for attachment of targeting agents, or 10 derivatized lipophilic compounds, such as lipid-derivatized glycopeptides of the invention.

[0336] Targeting mechanisms generally require that the targeting agents be positioned on the surface of the liposome in such a manner that the target moieties are available for interaction with the target, for example, a cell surface receptor. The carbohydrates of the invention may be attached to a lipid molecule before the liposome is formed using methods 15 known to those of skill in the art (e.g., alkylation or acylation of a hydroxyl group present on the carbohydrate with a long chain alkyl halide or with a fatty acid, respectively). Alternatively, the liposome may be fashioned in such a way that a connector portion is first incorporated into the membrane at the time of forming the membrane. The connector portion must have a lipophilic portion, which is firmly embedded and anchored in the membrane. It 20 must also have a reactive portion, which is chemically available on the aqueous surface of the liposome. The reactive portion is selected so that it will be chemically suitable to form a stable chemical bond with the targeting agent or carbohydrate, which is added later. In some cases it is possible to attach the target agent to the connector molecule directly, but in most instances it is more suitable to use a third molecule to act as a chemical bridge, thus linking 25 the connector molecule which is in the membrane with the target agent or carbohydrate which is extended, three dimensionally, off of the vesicle surface.

[0337] The compounds prepared by the methods of the invention may also find use as diagnostic reagents. For example, labeled compounds can be used to locate areas of inflammation or tumor metastasis in a patient suspected of having an inflammation. For this 30 use, the compounds can be labeled with  $^{125}\text{I}$ ,  $^{14}\text{C}$ , or tritium.

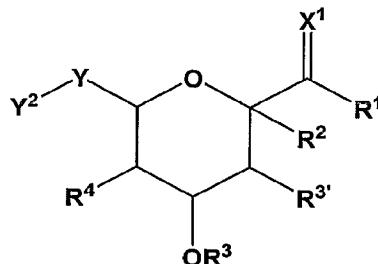
[0338] Moreover, the invention provides methods of preventing, curing or ameliorating a disease state by administering a conjugate of the invention to a subject at risk of developing

the disease or to a subject that has the disease. The conjugate is administered in a therapeutically effective amount. Because many of the conjugates, particularly those that include a polymeric modifying group, are anticipated to display enhanced in vivo residence times, a therapeutically effective dosage is readily determinable from a dosage of the non-  
5 conjugated therapeutic agent typically administered.

[0339] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent  
10 applications cited herein are hereby incorporated by reference in their entirety for all purposes.

**WHAT IS CLAIMED IS:**

1        1. A compound comprising a moiety represented by Formula I:



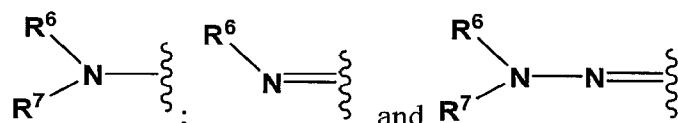
2  
3        wherein

4        X¹ is a member selected from substituted or unsubstituted alkyl, O and NR⁸  
5        wherein

6        R⁸ is a member selected from H, OH, substituted or unsubstituted alkyl and  
7        substituted or unsubstituted heteroalkyl;

8        Y is a member selected from CH₂, CH(OH)CH₂, CH(OH)CH(OH)CH₂, CH,  
9              CH(OH)CH or CH(OH)CH(OH)CH, CH(OH), CH(OH)CH(OH), and  
10          CH(OH)CH(OH)CH(OH);

11        Y² is a member selected from substituted or unsubstituted alkyl, R⁶, substituted or  
12        unsubstituted heteroalkyl



13        wherein

14        R⁶ and R⁷ are members independently selected from H, C(O)R⁶ᵇ, --Lᵃ--R⁶ᵇ,  
15              substituted or unsubstituted alkyl and substituted or unsubstituted  
16              heteroalkyl;

17        wherein

18              Lᵃ is a member selected from a bond and a linker group; and

19              R⁶ᵇ is a member selected from H and R⁶ᵃ

20        wherein

21              R⁶ᵃ is a modifying group

22        R¹ is a member selected from OR⁹, NR⁹R¹⁰, substituted or unsubstituted alkyl and  
23              substituted or unsubstituted heteroalkyl

24        wherein

26           R<sup>9</sup> and R<sup>10</sup> are members independently selected from H, substituted or  
27           unsubstituted alkyl, substituted or unsubstituted heteroalkyl, and  
28           C(O)R<sup>11</sup>

29           wherein

30           R<sup>11</sup> is selected from substituted or unsubstituted alkyl, substituted or  
31           unsubstituted heteroalkyl, substituted or unsubstituted aryl,  
32           substituted or unsubstituted heteroaryl and substituted or  
33           unsubstituted heterocycloalkyl;

34           R<sup>2</sup> is a member selected from a nucleotide, an activating moiety, an amino acid  
35           residue of a peptide, a carbohydrate moiety attached to an amino acid residue  
36           of a peptide, and a carbohydrate moiety attached to an amino acid residue of a  
37           peptide through a linker comprising at least a second carbohydrate moiety;

38           R<sup>3</sup> is a member selected from H, substituted or unsubstituted alkyl and substituted or  
39           unsubstituted heteroalkyl;

40           R<sup>3</sup>, and R<sup>4</sup> are members independently selected from H, OH, substituted or  
41           unsubstituted alkyl, substituted or unsubstituted heteroalkyl and NHC(O)R<sup>12</sup>

42           wherein

43           R<sup>12</sup> is a member selected from substituted or unsubstituted alkyl, substituted or  
44           unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted  
45           or unsubstituted heteroaryl, substituted or unsubstituted  
46           heterocycloalkyl and NR<sup>13</sup>R<sup>14</sup>

47           wherein

48           R<sup>13</sup> and R<sup>14</sup> are members independently selected from H, substituted or  
49           unsubstituted alkyl and substituted or unsubstituted heteroalkyl.

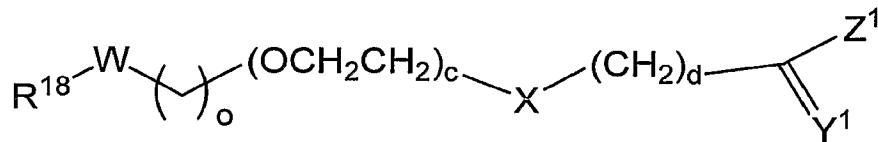
1           2.       The compound according to claim 1, wherein Y<sup>2</sup> comprises at least one  
2       modifying group.

1           3.       The compound according to claim 1, wherein R<sup>3</sup> is H.

1           4.       The compound according to claim 2, wherein at least one of R<sup>6</sup> and R<sup>7</sup>  
2       comprises a modifying group.

1           5.       The compound according to claim 2, wherein said modifying group is  
2       a member selected from linear- and branched-poly(ethylene glycol).

1                 **6.**         The compound according to claim **5**, wherein said PEG moiety is  
 2 linear PEG and said linear PEG has a structure according to the following formula:



3                 wherein

5                 R<sup>18</sup> is a member selected from H, substituted or unsubstituted alkyl, substituted or  
 6                 unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or  
 7                 unsubstituted heterocycloalkyl, substituted or unsubstituted heteroalkyl, *e.g.*,  
 8                 acetal, OHC-, H<sub>2</sub>N-CH<sub>2</sub>CH<sub>2</sub>-, HS-CH<sub>2</sub>CH<sub>2</sub>-, and-(CH<sub>2</sub>)<sub>q</sub>C(Y<sup>1</sup>)Z<sup>2</sup>; -sugar-  
 9                 nucleotide, and protein;

10                 c is an integer selected from 1 to 2500;

11                 d, o, and q are integers independently selected from 0 to 20;

12                 Z is a member selected from OH, NH<sub>2</sub>, halogen, S-R<sup>19</sup>, the alcohol portion of  
 13                 activated esters, -(CH<sub>2</sub>)<sub>d1</sub>C(Y<sup>3</sup>)V, -(CH<sub>2</sub>)<sub>d1</sub>U(CH<sub>2</sub>)<sub>g</sub>C(Y<sup>3</sup>)<sub>v</sub>, sugar-nucleotide,  
 14                 protein, and leaving groups, *e.g.*, imidazole, p-nitrophenyl, HOBT, tetrazole,  
 15                 and halide;

16                 X, Y<sup>1</sup>, Y<sup>3</sup>, W and U are independently selected from O, S, N-R<sup>20</sup>;

17                 V is a member selected from OH, NH<sub>2</sub>, halogen, S-R<sup>21</sup>, the alcohol component of  
 18                 activated esters, the amine component of activated amides, sugar-nucleotides,  
 19                 and proteins;

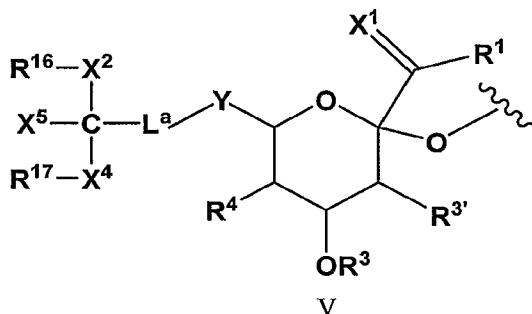
20                 d1, g and v are integers independently selected from 0 to 20; and

21                 R<sup>19</sup>, R<sup>20</sup> and R<sup>21</sup> are independently selected from H, substituted or unsubstituted alkyl,  
 22                 substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl,  
 23                 substituted or unsubstituted heterocycloalkyl and substituted or unsubstituted  
 24                 heteroaryl.

1                 **7.**         The compound according to claim **6**, wherein said linear PEG is  
 2 attached to a member selected from a carbohydrate moiety attached to an amino acid residue

3 of said peptide, a carbohydrate moiety attached to an amino acid residue of said peptide  
 4 through a linker comprising at least a second carbohydrate moiety.

1           **8.**       The compound according to claim 5, wherein said moiety has a  
 2 structure according to Formula V:



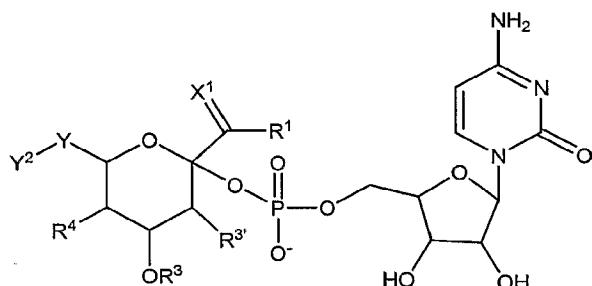
3           L<sup>a</sup> is a linker selected from a bond, substituted or unsubstituted alkyl and substituted  
 4           or unsubstituted heteroalkyl

5           R<sup>16</sup> and R<sup>17</sup> are independently selected polymeric arms;

6           X<sup>2</sup> and X<sup>4</sup> are independently selected linkage fragments joining polymeric moieties  
 7           R<sup>16</sup> and R<sup>17</sup> to C; and

8           X<sup>5</sup> is a non-reactive group.

1           **9.**       The compound according to claim 1 having the formula:



1           **10.**      The compound according to claim 1, wherein Y<sup>2</sup> is N(R<sup>6</sup>)-L<sup>a</sup>-(m-PEG)<sub>s</sub>

2           wherein

3           L<sup>a</sup> is a linker moiety which is a member selected from an amino acid residue  
 4           and a peptidyl residue; and

5           s is an integer from 1 to 3.

1                   **11.** A method of forming a covalent conjugate between a modified  
2 saccharyl fragment and a glycosylated or non-glycosylated peptide, said method comprising:  
3 enzymatically transferring said modified saccharyl fragment from an activated  
4 modified saccharyl fragment to an acceptor moiety on said peptide.

1                   **12.** The method according to claim 11, wherein said modified saccharyl  
2 fragment is covalently attached to a glycosyl residue covalently attached to said peptide.

1                   **13.** The method according to claim 11, wherein said modified saccharyl  
2 fragment is covalently attached to an amino acid residue of said peptide.

1                   **14.** The method of claim 11, wherein said enzyme is a glycosyltransferase  
2 which is a member selected from sialyl transferases, trans-sialidases, galactosyltransferases,  
3 glucosyltransferases, GalNAc transferase, GlcNAc transferase, fucosyl transferases, and  
4 mannosyltransferases.

1                   **15.** The method of claim 14, wherein said glycosyltransferase is  
2 recombinant.

1                   **16.** The method according to claim 11, wherein said method is performed  
2 in a cell-free environment.

1                   **17.** A pharmaceutical composition comprising a pharmaceutically  
2 acceptable carrier and a conjugate comprising a modified saccharyl fragment covalently  
3 linked to a glycosylated or non-glycosylated peptide.

1                   **18.** A composition for forming a conjugate between a peptide and a  
2 modified saccharyl fragment, said composition comprising: a mixture of an activated  
3 modified saccharyl fragment, an enzyme for which said activated modified saccharyl  
4 fragment is a substrate, and a peptide acceptor substrate, wherein said modified saccharyl  
5 fragment has covalently attached thereto a member selected from water-soluble polymers,  
6 therapeutic moieties and biomolecules.

|  |  |
|--|--|
| 12AP1/E5 -- Viventia Biotech               | AI-201 – AutoImmune                      |
| 1964 -- Aventis                            | AI-301 – AutoImmune                      |
| 20K growth hormone -- AMUR                 | AIDS vaccine – ANRS, CIBG, Hesed         |
| 28P6/E6 -- Viventia Biotech                | Biomed, Hollis-Eden, Rome, United        |
| 3-Hydroxyphthaloyl-beta-lactoglobulin –    | Biomedical, American Home Products,      |
| 4-IBB ligand gene therapy –                | Maxygen                                  |
| 64-Cu MAb conjugate TETA-1A3 --            | airway receptor ligand -- IC Innovations |
| Mallinckrodt Institute of Radiology        | AJvW 2 -- Ajinomoto                      |
| 64-Cu MAb conjugate TETA-cT84.66           | AK 30 NGF -- Alkermes                    |
| 64-Cu Trastuzumab TETA conjugate –         | Albuferon -- Human Genome Sciences       |
| Genentech                                  | albumin – Biogen, DSM Anti-Infectives,   |
| A 200 -- Amgen                             | Genzyme Transgenics, PPL Therapeutics,   |
| A10255 – Eli Lilly                         | TranXenoGen, Welfide Corp.               |
| A1PDX – Hederal TTherapeutics              | aldesleukin -- Chiron                    |
| A6 -- Angstrom                             | alefacept -- Biogen                      |
| aaAT-III -- Genzyme                        | Alemtuzumab –                            |
| Abciximab -- Centocor                      | Allergy therapy -- ALK-Abello/Maxygen,   |
| ABI.001 – Atlantic BioPharmaceuticals      | ALK-Abello/RP Scherer                    |
| ABT-828 – Abbott                           | allergy vaccines -- Allergy Therapeutics |
| Accutin                                    | Alnidofibatide -- Aventis Pasteur        |
| Actinohivin                                | Alnorine -- SRC VB VECTOR                |
| activin -- Biotech Australia, Human        | ALP 242 -- Gruenthal                     |
| Therapeutics                               | Alpha antitrypsin -- Arriva/Hyland       |
| activin -- Curis                           | Immuno/ProMetic/Protease Sciences        |
| AD 439 – Tanox                             | Alpha-1 antitrypsin – Cutter, Bayer, PPL |
| AD 519 – Tanox                             | Therapeutics, Profile, ZymoGenetics,     |
| Adalimumab -- Cambridge Antibody Tech.     | Arriva                                   |
| Adenocarcinoma vaccine – Biomira -- NIS    | Alpha-1 protease inhibitor -- Genzyme    |
| Adenosine A2B receptor antagonists --      | Transgenics, Welfide Corp.               |
| Adenosine Therapeutics                     | Alpha-galactose fusion protein –         |
| ADP-001 – Axis Genetics                    | Immunomedics                             |
| AF 13948 – Affymax                         | Alpha-galactosidase A -- Research        |
| Afelimomab – Knoll                         | Corporation Technologies                 |
| AFP-SCAN – Immunomedics                    | Alpha-glucosidase – Genzyme, Novazyme    |
| AG 2195 – Corixa                           | Alpha-lactalbumin                        |
| agalsidase alfa -- Transkaryotic Therapies | Alpha-L-iduronidase -- Transkaryotic     |
| agalsidase beta -- Genzyme                 | Therapies, BioMarin                      |
| AGENT – Antisoma                           | alteplase -- Genentech                   |
| AI 300 – AutoImmune                        | alvircept sudotox -- NIH                 |
| AI-101 – Teva                              | ALX1-11 –sNPS Pharmaceuticals            |
| AI-102 – Teva                              | Alzheimer's disease gene therapy –       |

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| AM-133 -- AMRAD  | Anti-B4 MAb-DC1 conjugate -- ImmunoGen                |
| Amb a 1 immunostim conj. -- Dynavax  | Anti-B7 antibody PRIMATIZED -- IDEC                   |
| AMD 3100 -- AnorMED -- NIS   | Anti-B7-1 MAb 16-10A1                                 |
| AMD 3465 -- AnorMED -- NIS   | Anti-B7-1 MAb 1G10                                    |
| AMD 3465 -- AnorMED -- NIS   | Anti-B7-2 MAb GL-1                                    |
| AMD Fab -- Genentech   | Anti-B7-2-gelonin immunotoxin --                      |
| Amediplase -- Menarini, Novartis   | Antibacterials/antifungals --                         |
| AM-F9  | Diversa/IntraBiotics                                  |
| Amoebiasis vaccine   | Anti-beta-amyloid monoclonal antibodies --            |
| Amphiregulin -- Octagene   | Cambridge Antibody Tech., Wyeth-Ayerst                |
| anakinra -- Amgen  | Anti-BLyS antibodies -- Cambridge                     |
| analgesic -- Nobex   | Antibody Tech. /Human Genome Sciences                 |
| ancestim -- Amgen  | Antibody-drug conjugates -- Seattle                   |
| AnergiX.RA -- Corixa, Organon  | Genetics/Eos  |
| Angiocidin -- InKine   | Anti-C5 MAb BB5-1 -- Alexion                          |
| angiogenesis inhibitors -- ILEX  | Anti-C5 MAb N19-8 -- Alexion                          |
| AngioMab -- Antisoma   | Anti-C8 MAb   |
| Angiopoietins -- Regeneron/Procter & Gamble                                  | anticancer cytokines -- BioPulse                      |
| angiostatin -- EntreMed  | anticancer matrix -- Telios Integra                   |
| Angiostatin/endostatin gene therapy -- Genetix Pharmaceuticals               | Anticancer monoclonal antibodies -- ARIUS, Immunex    |
| angiotensin-II, topical -- Maret   | anticancer peptides -- Maxygen, Micrologix            |
| Anthrax -- EluSys Therapeutics/US Army Medical Research Institute            | Anticancer prodrug Tech. -- Alexion                   |
| Anthrax vaccine  | Antibody Technologies                                 |
| Anti platelet-derived growth factor D human monoclonal antibodies -- CuraGen | anticancer Troy-Bodies -- Affite -- Affitech          |
| Anti-17-1A MAb 3622W94 -- GlaxoSmithKline                                    | anticancer vaccine -- NIH                             |
| Anti-2C4 MAb -- Genentech  | anticancers -- Epimmune                               |
| anti-4-1BB monoclonal antibodies -- Bristol- Myers Squibb                    | Anti-CCR5/CXCR4 sheep MAb -- KS Biomedix Holdings     |
| Anti-Adhesion Platform Tech. -- Cytovax                                      | Anti-CD11a MAb KBA --                                 |
| Anti-adipocyte MAb -- Cambridge Antibody Tech./ObeSys                        | Anti-CD11a MAb M17                                    |
| antiallergics -- Maxygen   | Anti-CD11a MAb TA-3 --                                |
| antiallergy vaccine -- Acambis   | Anti-CD11a MAb WT.1 --                                |
| Anti-alpha-4-integrin MAb  | Anti-CD11b MAb -- Pharmacia                           |
| Anti-angiogenesis monoclonal antibodies -- KS Biomedix/Schering AG           | Anti-CD11b MAb LM2                                    |
|  | Anti-CD154 MAb -- Biogen                              |
|  | Anti-CD16-anti-CD30 MAb -- Biotest                    |
|  | Anti-CD18 MAb -- Pharmacia                            |
|  | Anti-CD19 MAb B43 --                                  |
|  | Anti-CD19 MAb -liposomal sodium butyrate conjugate -- |

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| Anti-CD19 MAb-saporin conjugate –                            | Anti-CD4 MAb KT6  |
| Anti-CD19-dsFv-PE38-immunotoxin –                            | Anti-CD4 MAb OX38   |
| Anti-CD2 MAb 12-15 –   | Anti-CD4 MAb PAP conjugate -- Bristol-Myers Squibb                  |
| Anti-CD2 MAb B-E2 -- Diaclone                                | Anti-CD4 MAb RIB 5-2  |
| Anti-CD2 MAb OX34 –  | Anti-CD4 MAb W3/25  |
| Anti-CD2 MAb OX54 –  | Anti-CD4 MAb YTA 3.1.2  |
| Anti-CD2 MAb OX55 –  | Anti-CD4 MAb YTS 177-9  |
| Anti-CD2 MAb RM2-1   | Anti-CD40 ligand MAb 5c8 -- Biogen                                  |
| Anti-CD2 MAb RM2-2   | Anti-CD40 MAb   |
| Anti-CD2 MAb RM2-4   | Anti-CD40 MAb 5D12 – Tanox  |
| Anti-CD20 MAb BCA B20  | Anti-CD44 MAb A3D8  |
| Anti-CD20-anti-Fc alpha RI bispecific MAb – Medarex, Tenovus | Anti-CD44 MAb GKWA3   |
| Anti-CD22 MAb-saporin-6 complex –                            | Anti-CD44 MAb IM7   |
| Anti-CD3 immunotoxin –                                       | Anti-CD44 MAb KM81  |
| Anti-CD3 MAb 145-2C11 -- Pharming                            | Anti-CD44 variant monoclonal antibodies -- Corixa/Hebrew University |
| Anti-CD3 MAb CD4IgG conjugate -- Genentech                   | Anti-CD45 MAb BC8-I-131   |
| Anti-CD3 MAb humanised – Protein Design, RW Johnson          | Anti-CD45RB MAb   |
| Anti-CD3 MAb WT32  | Anti-CD48 MAb HuLy-m3   |
| Anti-CD3 MAb-ricin-chain-A conjugate –                       | Anti-CD48 MAb WM-63   |
| Anti-CD3 MAb-xanthine-oxidase conjugate –                    | Anti-CD5 MAb -- Becton Dickinson                                    |
| Anti-CD30 MAb BerH2 -- Medac                                 | Anti-CD5 MAb OX19   |
| Anti-CD30 MAb-saporin conjugate                              | Anti-CD6 MAb  |
| Anti-CD30-scFv-ETA'-immunotoxin                              | Anti-CD7 MAb-PAP conjugate  |
| Anti-CD38 MAb AT13/5   | Anti-CD7 MAb-ricin-chain-A conjugate                                |
| Anti-CD38 MAb-saporin conjugate                              | Anti-CD8 MAb – Amerimmune, Cytodyn, Becton Dickinson                |
| Anti-CD3-anti-CD19 bispecific MAb                            | Anti-CD8 MAb 2-43   |
| Anti-CD3-anti-EGFR MAb                                       | Anti-CD8 MAb OX8  |
| Anti-CD3-anti-interleukin-2-receptor MAb                     | Anti-CD80 MAb P16C10 -- IDEC  |
| Anti-CD3-anti-MOV18 MAb -- Centocor                          | Anti-CD80 MAb P7C10 -- ID Vaccine                                   |
| Anti-CD3-anti-SCLC bispecific MAb                            | Anti-CD8-idarubicin conjugate                                       |
| Anti-CD4 idiotype vaccine                                    | Anti-CEA MAb CE-25  |
| Anti-CD4 MAb – Centocor, IDEC Pharmaceuticals, Xenova Group  | Anti-CEA MAb MN 14 – Immunomedics                                   |
| Anti-CD4 MAb 16H5  | Anti-CEA MAb MN14-PE40 conjugate – Immunomedics                     |
| Anti-CD4 MAb 4162W94 -- GlaxoSmithKline                      | Anti-CEA MAb T84.66-interleukin-2 conjugate                         |
| Anti-CD4 MAb B-F5 -- Diaclone                                | Anti-CEA sheep MAb -- KS Biomedix Holdings                          |
| Anti-CD4 MAb GK1-5   |   |

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| Anti-cell surface monoclonal antibodies -- Cambridge Antibody Tech. /Pharmacia | Anti-HIV antibody -- Epocyte   |
| Anti-c-erbB2-anti-CD3 bifunctional MAb -- Otsuka                               | anti-HIV catalytic antibody -- Hesed Biomed  |
| Anti-CMV MAb -- Scotgen  | anti-HIV fusion protein -- Idun  |
| Anti-CTLA-4 MAb  | anti-HIV proteins -- Cangene   |
| Anti-EGFR catalytic antibody -- Hesed Biomed                                   | Anti-HM1-24 MAb -- Chugai  |
| anti-EGFR immunotoxin -- IVAX  | Anti-hR3 MAb   |
| Anti-EGFR MAb -- Abgenix   | Anti-Human-Carcinoma-Antigen MAb -- Epocyte  |
| Anti-EGFR MAb 528  | Anti-ICAM-1 MAb -- Boehringer Ingelheim  |
| Anti-EGFR MAb KSB 107 -- KS Biomedix   | Anti-ICAM-1 MAb 1A-29 -- Pharmacia   |
| Anti-EGFR MAb-DM1 conjugate -- ImmunoGen                                       | Anti-ICAM-1 MAb HA58   |
| Anti-EGFR MAb-LA1 --   | Anti-ICAM-1 MAb YN1/1.7.4  |
| Anti-EGFR sheep MAb -- KS Biomedix   | Anti-ICAM-3 MAb ICM3 -- ICOS   |
| Anti-FAP MAb F19-I-131   | Anti-idiotype breast cancer vaccine 11D10  |
| Anti-Fas IgM MAb CH11  | Anti-idiotype breast cancer vaccine ACA14C5 --                                       |
| Anti-Fas MAb Jo2   | Anti-idiotype cancer vaccine -- ImClone Systems/Merck KGaA ImClone, Viventia Biotech |
| Anti-Fas MAb RK-8  | Anti-idiotype cancer vaccine 1A7 -- Titan  |
| Anti-Flt-1 monoclonal antibodies -- ImClone                                    | Anti-idiotype cancer vaccine 3H1 -- Titan  |
| Anti-fungal peptides -- State University of New York                           | Anti-idiotype cancer vaccine TriAb -- Titan  |
| antifungal tripeptides -- BTG  | Anti-idiotype Chlamydia trachomatis vaccine  |
| Anti-ganglioside GD2 antibody-interleukin-2 fusion protein -- Lexigen          | Anti-idiotype colorectal cancer vaccine -- Novartis                                  |
| Anti-GM2 MAb -- Kyowa  | Anti-idiotype colorectal cancer vaccine -- Onyxax                                    |
| Anti-GM-CSF receptor monoclonal antibodies -- AMRAD                            | Anti-idiotype melanoma vaccine -- IDEC Pharmaceuticals                               |
| Anti-gp130 MAb -- Tosoh  | Anti-idiotype ovarian cancer vaccine ACA 125   |
| Anti-HCA monoclonal antibodies -- AltaRex/Epigen                               | Anti-idiotype ovarian cancer vaccine AR54 - - AltaRex                                |
| Anti-hCG antibodies -- Abgenix/AVI BioPharma                                   | Anti-idiotype ovarian cancer vaccine CA- 125 -- AltaRex, Biomira                     |
| Anti-heparanase human monoclonal antibodies -- Oxford Glycosciences/Medarex    | Anti-IgE catalytic antibody -- Hesed Biomed  |
| Anti-hepatitis C virus human monoclonal antibodies -- XTL Biopharmaceuticals   | Anti-IgE MAb E26 -- Genentech  |
| Anti-HER-2 antibody gene therapy   | Anti-IGF-1 MAb   |
| Anti-herpes antibody -- Epocyte  | anti-inflammatory -- GeneMax   |
|  | anti-inflammatory peptide -- BTG   |

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| anti-integrin peptides -- Burnha   | Anti-mu MAb -- Novartis  |
| Anti-interferon-alpha-receptor MAb 64G12 -- Anti-MUC-1 MAb                                 |  |
| Pharma Pacific Management  | Anti-Nogo-A MAb IN1  |
| Anti-interferon-gamma MAb -- Protein Design Labs   | Anti-nuclear autoantibodies -- Procyon   |
| Anti-interferon-gamma polyclonal antibody - - Advanced Biotherapy                          | Anti-ovarian cancer monoclonal antibodies - - Dompe  |
| Anti-interleukin-10 MAb --   | Anti-p185 monoclonal antibodies  |
| Anti-interleukin-12 MAb --   | Anti-p43 MAb   |
| Anti-interleukin-1-beta polyclonal antibody -- R&D Systems                                 | Antiparasitic vaccines   |
| Anti-interleukin-2 receptor MAb 2A3  | Anti-PDGF/bFGF sheep MAb -- KS Biomedix  |
| Anti-interleukin-2 receptor MAb 33B3-1 -- Immunotech                                       | Anti-properdin monoclonal antibodies -- Abgenix/Gliatech                                   |
| Anti-interleukin-2 receptor MAb ART-18   | Anti-PSMA MAb J591 -- BZL Biologics  |
| Anti-interleukin-2 receptor MAb LO-Tact-1  | Anti-Rev MAb gene therapy --   |
| Anti-interleukin-2 receptor MAb Mikbeta1   | Anti-RSV antibodies -- Epicyte, Intracell  |
| Anti-interleukin-2 receptor MAb NDS61  | Anti-RSV monoclonal antibodies -- Medarex/MedImmune, Applied Molecular Evolution/MedImmune |
| Anti-interleukin-4 MAb 11B11   | Anti-RSV MAb, inhalation -- Alkermes/MedImmune   |
| Anti-interleukin-5 MAb -- Wallace Laboratories   | Anti-RT gene therapy   |
| Anti-interleukin-6 MAb -- Centocor, Diaclone, Pharmadigm                                   | Antisense K-ras RNA gene therapy   |
| Anti-interleukin-8 MAb -- Xenotech   | Anti-SF-25 MAb   |
| Anti-JL1 MAb   | Anti-sperm antibody -- Epicyte   |
| Anti-Klebsiella sheep MAb -- KS Biomedix Holdings  | Anti-Tac(Fv)-PE38 conjugate  |
| Anti-Laminin receptor MAb-liposomal doxorubicin conjugate                                  | Anti-TAPA/CD81 MAb AMP1  |
| Anti-LCG MAb -- Cytoclonal   | Anti-tat gene therapy  |
| Anti-lipopolysaccharide MAb -- VitaResc  | Anti-TCR-alphabeta MAb H57-597   |
| Anti-L-selectin monoclonal antibodies -- Protein Design Labs, Abgenix, Stanford University | Anti-TCR-alphabeta MAb R73   |
| Anti-MBL monoclonal antibodies -- Alexion/Brigham and Women's Hospital                     | Anti-tenascin MAb BC-4-I-131   |
| Anti-MHC monoclonal antibodies   | Anti-TGF-beta human monoclonal antibodies -- Cambridge Antibody Tech., Genzyme             |
| Anti-MIF antibody humanised -- IDEC, Cytokine PharmaSciences                               | Anti-TGF-beta MAb 2G7 -- Genentech   |
| Anti-MRSA/VRSA sheep MAb -- KS Biomedix Holdings   | Antithrombin III -- Genzyme Transgenics, Aventis, Bayer, Behringwerke, CSL, Myriad         |
|  | Anti-Thy1 MAb  |
|  | Anti-Thy1.1 MAb  |

Anti-tissue factor/factor VIIA sheep MAb -- ARGENT gene delivery systems -- ARIAD  
 KS Biomedix Arresten  
 Anti-TNF monoclonal antibodies -- ART-123 -- Asahi Kasei  
 Centocor, Chiron, Peptech, Pharacia, arylsulfatase B -- BioMarin  
 Serono Arylsulfatase B, Recombinant human --  
 Anti-TNF sheep MAb -- KS Biomedix BioMarin  
 Holdings AS 1051 -- Ajinomoto  
 Anti-TNFAalpha MAb -- Genzyme ASI-BCL -- Intracell  
 Anti-TNFAalpha MAb B-C7 -- Diaclone ATL-101 -- Alizyme  
 Anti-tooth decay MAb -- Planet BioTech. atrial natriuretic peptide -- Pharis  
 antitumour RNases -- NIH Aurintricarboxylic acid-high molecular  
 weight  
 Anti-VCAM MAb 2A2 -- Alexion autoimmune disorders -- GPC  
 Anti-VCAM MAb 3F4 -- Alexion Biotech/MorphoSys  
 Anti-VCAM-1 MAb Autoimmune disorders and transplant  
 Anti-VEC MAb -- ImClone rejection -- Bristol-Myers Squibb/Genzyme  
 Anti-VEGF MAb -- Genentech Tra  
 Anti-VEGF MAb 2C3 Autoimmune disorders/cancer --  
 Anti-VEGF sheep MAb -- KS Biomedix Abgenix/Chiron, /CuraGen  
 Holdings Autotaxin  
 Anti-VLA-4 MAb HP1/2 -- Biogen Avicidin -- NeoRx  
 Anti-VLA-4 MAb PS/2 axogenesis factor-1 -- Boston Life Sciences  
 Anti-VLA-4 MAb R1-2 Axokine -- Regeneron  
 Anti-VLA-4 MAb TA-2 B cell lymphoma vaccine -- Biomира  
 Anti-VRE sheep MAb -- KS Biomedix B7-1 gene therapy --  
 Holdings BABS proteins -- Chiron  
 ANUP -- TranXenoGen BAM-002 -- Novelos Therapeutics  
 ANUP-1 -- Pharis Bay-16-9996 -- Bayer  
 AOP-RANTES -- Senetek Bay-39-9437 -- Bayer  
 Apan-CH -- Praecis Pharmaceuticals Bay-50-4798 -- Bayer  
 APC-8024 -- Demegen BB-10153 -- British Biotech  
 ApoA-1 -- Milano, Pharmacia BBT-001 -- Bolder BioTech.  
 Apogen -- Alexion BBT-002 -- Bolder BioTech.  
 apolipoprotein A1 -- Avanir BBT-003 -- Bolder BioTech.  
 Apolipoprotein E -- Bio-Tech. General BBT-004 -- Bolder BioTech.  
 Applaggin -- Biogen BBT-005 -- Bolder BioTech.  
 aprotinin -- ProdiGene BBT-006 -- Bolder BioTech.  
 APT-070C -- AdProTech BBT-007 -- Bolder BioTech.  
 AR 177 -- Aronex Pharmaceuticals BCH-2763 -- Shire  
 AR 209 -- Aronex Pharmaceuticals, BCSF -- Millenium Biologix  
 Antigenics BDNF -- Regeneron -- Amgen  
 AR545C

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| Becaplermin -- Johnson & Johnson, Chiron   | BST-3002 -- BioStratum   |
| Bectumomab -- Immunomedics   | BTI 322 --   |
| Beta-adrenergic receptor gene therapy -- University of Arkansas  | butyrylcholinesterase -- Shire   |
| BI 51013 -- Behringwerke AG  | C 6822 -- COR Therapeutics   |
| BIBH 1 -- Boehringer Ingelheim   | C1 esterase inhibitor -- Pharming  |
| BIM-23190 -- Beaufour-Ipsen  | C3d adjuvant -- AdProTech  |
| birch pollen immunotherapy -- Pharmacia  | CAB-2.1 -- Millennium  |
| bispecific fusion proteins -- NIH  | calcitonin -- Inhale Therapeutics Systems, Aventis, Genetronics, TranXenoGen, Unigene, Rhone Poulenc Rohrer  |
| Bispecific MAb 2B1 -- Chiron   | calcitonin -- oral -- Nobex, Emisphere, Pharmaceutical Discovery   |
| Bitistatin   | Calcitonin gene-related peptide -- Asahi Kasei -- Unigene  |
| BIWA 4 -- Boehringer Ingelheim   | calcitonin, human -- Suntory   |
| blood substitute -- Northfield, Baxter Intl.   | calcitonin, nasal -- Novartis, Unigene   |
| BLP-25 -- Biomira  | calcitonin, Panoderm -- Elan   |
| BLS-0597 -- Boston Life Sciences   | calcitonin, Peptitrol -- Shire   |
| BLyS -- Human Genome Sciences  | calcitonin, salmon -- Therapicon   |
| BLyS radiolabelled -- Human Genome Sciences  | calin -- Biopharm  |
| BM 06021 -- Boehringer Mannheim  | Calphobindin I   |
| BM-202 -- BioMarin   | calphobindin I -- Kowa   |
| BM-301 -- BioMarin   | calreticulin -- NYU  |
| BM-301 -- BioMarin   | Campath-1G   |
| BM-302 -- BioMarin   | Campath-1M   |
| BMP 2 -- Genetics Institute/Medtronic-Sofamor Danek, Genetics Institute/Collagenesis, Genetics Institute/Yamanouch | cancer therapy -- Cangene  |
| BMP 2 gene therapy   | cancer vaccine -- Aixlie, Aventis Pasteur, Center of Molecular Immunology ,YM BioSciences, Cytos, Genzyme, Transgenics, Globelimmune, Igeneon, ImClone, Virogenetics, InterCell, Iomai, Jenner Biotherapies, Memorial Sloan-Kettering Cancer Center, Sydney Kimmel Cancer Center, Novavax, Protein Sciences, Argonex, SIGA |
| BMP 52 -- Aventis Pasteur, Biopharm  | Cancer vaccine ALVAC-CEA B7.1 -- Aventis Pasteur/Terion Biologics  |
| BMP-2 -- Genetics Institute  | Cancer vaccine CEA-TRICOM -- Aventis Pasteur/Terion Biologics  |
| BMS 182248 -- Bristol-Myers Squibb   | Cancer vaccine gene therapy -- Cantab Pharmaceuticals  |
| BMS 202448 -- Bristol-Myers Squibb   |  |
| bone growth factors -- IsoTis  |  |
| BPC-15 -- Pfizer   |  |
| brain natriuretic peptide --   |  |
| Breast cancer -- Oxford GlycoSciences/Medarex  |  |
| Breast cancer vaccine -- Therion Biologics, Oregon   |  |
| BSSL -- PPL Therapeutics   |  |
| BST-2001 -- BioStratum   |  |

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| Cancer vaccine HER-2/neu -- Corixa                     | CETP vaccine -- Avant  |
| Cancer vaccine THERATOPE -- Biomира                    | Cetrorelix   |
| cancer vaccine, PolyMASC -- Valentis                   | Cetuximab  |
| Candida vaccine -- Corixa, Inhibitex                   | CGH 400 -- Novartis  |
| Canstatin -- ILEX                                      | CGP 42934 -- Novartis  |
| CAP-18 -- Panorama                                     | CGP 51901 -- Tanox   |
| Cardiovascular gene therapy -- Collateral Therapeutics | CGRP -- Unigene  |
| carperitide -- Suntory                                 | CGS 27913 -- Novartis  |
| Casocidin-1 -- Pharis                                  | CGS 32359 -- Novartis  |
| CAT 152 -- Cambridge Antibody Tech.                    | Chagas disease vaccine -- Corixa                                       |
| CAT 192 -- Cambridge Antibody Tech.                    | chemokines -- Immune Response  |
| CAT 213 -- Cambridge Antibody Tech.                    | CHH 380 -- Novartis  |
| Catalase-- Enzon                                       | chitinase -- Genzyme, ICOS   |
| Cat-PAD -- Circassia                                   | Chlamydia pneumoniae vaccine -- Antex Biologics                        |
| CB 0006 -- Celltech                                    | Chlamydia trachomatis vaccine -- Antex Biologics                       |
| CCK(27-32)-- Akzo Nobel                                | Chlamydia vaccine -- GlaxoSmithKline                                   |
| CCR2-64I -- NIH  | Cholera vaccine CVD 103-HgR -- Swiss Serum and Vaccine Institute Berne |
| CD, Procept -- Palgent                                 | Cholera vaccine CVD 112 -- Swiss Serum and Vaccine Institute Berne     |
| CD154 gene therapy                                     | Cholera vaccine inactivated oral -- SBL Vaccin                         |
| CD39 -- Immunex  | Chrysalin -- Chrysalis BioTech.  |
| CD39-L2 -- Hyseq                                       | CI-782 -- Hitachi Kase   |
| CD39-L4 -- Hyseq                                       | Ciliary neurotrophic factor -- Fidia, Roche                            |
| CD4 fusion toxin -- Senetek                            | CIM project -- Active Biotech  |
| CD4 IgG -- Genentech                                   | CL 329753 -- Wyeth-Ayerst  |
| CD4 receptor antagonists -- Pharmacopeia/Progenics     | CL22, Cobra -- ML Laboratories   |
| CD4 soluble -- Progenics                               | Clenoliximab -- IDEC   |
| CD4, soluble -- Genzyme Transgenics                    | Clostridium difficile antibodies -- Epicycle                           |
| CD40 ligand -- Immunex                                 | clotting factors -- Octagene   |
| CD4-ricin chain A -- Genentech                         | CMB 401 -- Celltech  |
| CD59 gene therapy -- Alexion                           | CNTF -- Sigma-Tau  |
| CD8 TIL cell therapy -- Aventis Pasteur                | Cocaine abuse vaccine -- Cantab, ImmuLogic, Scripps                    |
| CD8, soluble -- Avidex                                 | coccidiomycosis vaccine -- Arizo                                       |
| CD95 ligand -- Roche                                   | collagen -- Type I -- Pharming   |
| CDP 571 -- Celltech                                    | Collagen formation inhibitors -- FibroGen                              |
| CDP 850 -- Celltech                                    |  |
| CDP 870 -- Celltech                                    |  |
| CDS-1 -- Ernest Orlando                                |  |
| Cedelizumab -- Ortho-McNeil                            |  |
| Cetermin -- Insmed                                     |  |

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| Collagen/hydroxyapatite/bone growth factor CY 1747 -- Epimmune |  |
| -- Aventis Pasteur, Biopharm, Orquest                          | CY 1748 -- Epimmune                        |
| collagenase -- BioSpecifics                                    | Cyanovirin-N                               |
| Colorectal cancer vaccine -- Wistar Institute                  | Cystic fibrosis therapy -- CBR/IVAX        |
| Component B, Recombinant -- Serono                             | CYT 351                                    |
| Connective tissue growth factor inhibitors --                  | cytokine Traps -- Regeneron                |
| FibroGen/Taisho  | cytokines -- Enzon, Cytoclonal             |
| Contortrostatin  | Cytomegalovirus glycoprotein vaccine --    |
| contraceptive vaccine -- Zonagen                               | Chiron, Aquila Biopharmaceuticals,         |
| Contraceptive vaccine hCG                                      | Aventis Pasteur, Virogenetics              |
| Contraceptive vaccine male reversible --                       | Cytomegalovirus vaccine live -- Aventis    |
| IMMUCON  | Pasteur                                    |
| Contraceptive vaccine zona pellucida --                        | Cytosine deaminase gene therapy --         |
| Zonagen  | GlaxoSmithKline                            |
| Copper-64 labelled MAb TETA-1A3 -- NCI                         | DA-3003 -- Dong-A                          |
| Coralyne   | DAB389interleukin-6 -- Senetek             |
| Corsevin M   | DAB389interleukin-7                        |
| C-peptide analogues -- Schwarz                                 | DAMP <sup>^</sup> -- Incyte Genomics       |
| CPI-1500 -- Consensus  | Daniprestim -- Pharmacia                   |
| CRF -- Neurobiological Tech.                                   | darbepoetin alfa -- Amgen                  |
| cRGDFV pentapeptide --   | DBI-3019 -- Diabetogen                     |
| CRL 1095 -- CytRx  | DCC -- Genzyme                             |
| CRL 1336 -- CytRx  | DDF -- Hyseq                               |
| CRL 1605 -- CytRx  | decorin -- Integra, Telios                 |
| CS-560 -- Sankyo   | defensins -- Large Scale Biology           |
| CSF -- ZymoGenetics  | DEGR-VIIa                                  |
| CSF-G -- Hangzhou, Dong-A, Hanmi                               | Delimmunised antibody 3B6/22 AGEN          |
| CSF-GM -- Cangene, Hunan, LG Chem                              | Deimmunised anti-cancer antibodies --      |
| CSF-M -- Zarix   | Biovation/Viragen                          |
| CT 1579 -- Merck Frosst  | Dendroamide A                              |
| CT 1786 -- Merck Frosst  | Dengue vaccine -- Bavarian Nordic, Merck   |
| CT-112 <sup>^</sup> -- BTG                                     | denileukin diftitox -- Ligand              |
| CTB-134L -- Xenova   | DES-1101 -- Desmos                         |
| CTC-111 -- Kaketsuken  | desirudin -- Novartis                      |
| CTGF -- FibroGen   | desmopressin -- Unigene                    |
| CTLA4-Ig -- Bristol-Myers Squibb                               | Desmoteplase -- Merck, Schering AG         |
| CTLA4-Ig gene therapy --                                       | Destabilase                                |
| CTP-37 -- AVI BioPharma  | Diabetes gene therapy -- DeveloGen, Pfizer |
| C-type natriuretic peptide -- Suntory                          | Diabetes therapy -- Crucell                |
| CVS 995 -- Corvas Intl.  | Diabetes type 1 vaccine -- Diamyd          |
| CX 397 -- Nikko Kyodo  | Therapeutics                               |

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| DiaCIM -- YM BioSciences  | EGF-P64k vaccine -- Center of Molecular Immunology                        |
| dialytic oligopeptides -- Research Corp                             | EL 246 -- LigoCyte  |
| Diamyd -- Diamyd Therapeutics                                       | elastase inhibitor -- Synergen  |
| DiaPep227-- Pepgen  | elcatonin -- Therapicon   |
| DiavaX -- Corixa  | EMD 72000 -- Merck KGaA   |
| Diphtheria tetanus pertussis-hepatitis B vaccine -- GlaxoSmithKline | Emdogain -- BIORA   |
| DIR therapy -- Solis Therapeutics --                                | emfliermin -- AMRAD   |
| DNase -- Genentech  | Emoctakin -- Novartis   |
| Dornase alfa -- Genentech   | enamel matrix protein -- BIORA  |
| Dornase alfa, inhalation -- Genentech                               | Endo III -- NYU   |
| Doxorubicin-anti-CEA MAb conjugate -- Immunomedics                  | endostatin -- EntreMed, Pharis  |
| DP-107 -- Trimeris  | Enhancins -- Micrologix   |
| drotrecogin alfa -- Eli Lilly                                       | Enlimomab -- Isis Pharm.  |
| DTctGMCSF   | Enoxaparin sodium -- Pharmuka   |
| DTP-polio vaccine -- Aventis Pasteur                                | enzyme linked antibody nutrient depletion therapy -- KS Biomedix Holdings |
| DU 257-KM231 antibody conjugate -- Kyowa                            | Eosinophil-derived neutralizing agent --                                  |
| dural graft matrix -- Integra                                       | EP-51216 -- Asta Medica   |
| Duteplase -- Baxter Intl.   | EP-51389 -- Asta Medica   |
| DWP-401 -- Daewoong   | EPH family ligands -- Regeneron   |
| DWP-404 -- Daewoong   | Epidermal growth factor -- Hitachi Kasei, Johnson & Johnson               |
| DWP-408 -- Daewoong   | Epidermal growth factor fusion toxin -- Senetek                           |
| E coli O157 vaccine -- NIH  | Epidermal growth factor-genistein --                                      |
| E21-R -- BresaGen   | EPI-HNE-4 -- Dyax   |
| Eastern equine encephalitis virus vaccine --                        | EPI-KAL2 -- Dyax  |
| Echicetin --  | Epoetin-alfa -- Amgen, Dragon Pharmaceuticals, Nanjing Huixin             |
| Echinhibin 1 --   | Epratuzumab -- Immunomedics   |
| Echistatin -- Merck   | Epstein-Barr virus vaccine -- Aviron/SmithKline Beecham, Bioresearch      |
| Echitamine --   | Eptacog alfa -- Novo Nordisk  |
| EC-SOD -- PPL Therapeutics  | Eptifibatide -- COR Therapeutics  |
| EDF -- Ajinomoto  | erb-38 --   |
| EDN derivative -- NIH   | Erlizumab -- Genentech  |
| EDNA -- NIH   |   |
| Edobacomab -- XOMA  |   |
| Edrecolomab -- Centocor   |   |
| EF 5077   |   |
| Efalizumab -- Genentech   |   |
| EGF fusion toxin -- Seragen, Ligand                                 |   |

erythropoietin -- Alkermes, ProLease, Dong-Fas TR -- Human Genome Sciences  
 A, Elanex, Genetics Institute, LG Chem,  
 Protein Sciences, Serono, Show Brand,  
 SRC VB VECTOR, Transkaryotic  
 Therapies  
 Erythropoietin Beta -- Hoffman La Roche  
 Erythropoietin/Epoetin alfa -- Chugai  
 Escherichia coli vaccine -- North American  
 Vaccine, SBL Vaccin, Swiss Serum and  
 Vaccine Institute Berne  
 etanercept -- Immunex  
 examorelin -- Mediolanum  
 exonuclease VII  
 F 105 -- Centocor  
 F-992 -- Fornix  
 Factor IX -- Alpha Therapeutics, Welfide  
 Corp., CSL, genetics Institute/AHP,  
 Pharmacia, PPL Therapeutics  
 Factor IX gene therapy -- Cell Genesys  
 Factor VII -- Novo Nordisk, Bayer, Baxter  
 Intl.  
 Factor VIIa -- PPL Therapeutics,  
 ZymoGenetics  
 Factor VIII -- Bayer Genentech, Beaufour-  
 Ipsen, CLB, Inex, Octagen, Pharmacia,  
 Pharming  
 Factor VIII -- PEGylated -- Bayer  
 Factor VIII fragments -- Pharmacia  
 Factor VIII gene therapy -- Targeted  
 Genetics  
 Factor VIII sucrose formulation -- Bayer,  
 Genentech  
 Factor VIII-2 -- Bayer  
 Factor VIII-3 -- Bayer  
 Factor Xa inhibitors -- Merck, Novo Nordisk, Ganirelix -- Roche  
 Mochida  
 Factor XIII -- ZymoGenetics  
 Factors VIII and IX gene therapy -- Genetics Institute/Targeted Genetics  
 Famoxin -- Genset  
 Fas (delta) TM protein -- LXR BioTech.  
 Felizumab -- Scotgen  
 FFR-VIIa -- Novo Nordisk  
 FG-001 -- F-Gene  
 FG-002 -- F-Gene  
 FG-004 -- F-Gene  
 FG-005 -- F-Gene  
 FGF + fibrin -- Repair  
 Fibrimage -- Bio-Tech. General  
 fibrin-binding peptides -- ISIS Innovation  
 fibrinogen -- PPL Therapeutics, Pharming  
 fibroblast growth factor -- Chiron, NYU,  
 Ramot, ZymoGenetics  
 fibrolase conjugate -- Schering AG  
 Filgrastim -- Amgen  
 filgrastim -- PDA modified -- Xencor  
 FLT-3 ligand -- Immunex  
 FN18 CRM9 --  
 follistatin -- Biotech Australia, Human  
 Therapeutics  
 follitropin alfa -- Alkermes, ProLease,  
 PowderJect, Serono, Akzo Nobel  
 Follitropin Beta -- Bayer, Organon  
 FP 59  
 FSH -- Ferring  
 FSH + LH -- Ferring  
 F-spondin -- CeNeS  
 fusion protein delivery system -- UAB  
 Research Foundation  
 fusion toxins -- Boston Life Sciences  
 G 5598 -- Genentech  
 GA-II -- Transkaryotic Therapies  
 Gamma-interferon analogues -- SRC VB  
 VECTOR  
 gastric lipase -- Meristem  
 Gavilimomab --  
 G-CSF -- Amgen, SRC VB VECTOR  
 GDF-1 -- CeNeS  
 GDF-5 -- Biopharm  
 GDNF -- Amgen

gelsolin -- Biogen  
 Gemtuzumab ozogamicin -- Celltech  
 Gene-activated epoetin-alfa -- Aventis  
     Pharma -- Transkaryotic Therapies  
 Glanzmann thrombasthenia gene therapy --  
 Glatiramer acetate -- Yeda  
 glial growth factor 2 -- CeNeS  
 GLP-1 -- Amylin, Suntory, TheraTech,  
     Watson  
 GLP-1 peptide analogues -- Zealand  
     Pharaceuticals  
 glucagon -- Eli Lilly, ZymoGenetics  
 Glucagon-like peptide-1 7-36 amide --  
     Suntory  
 Glucocerebrosidase -- Genzyme  
 glutamate decarboxylase -- Genzyme  
     Transgenics  
 Glycoprotein S3 -- Kureha  
 GM-CSF -- Immunex  
 GM-CSF tumour vaccine -- PowderJect  
 GnRH immunotherapeutic -- Protherics  
 gp75 antigen -- ImClone  
 gp96 -- Antigenics  
 GPI 0100 -- Galenica  
 GR 4991W93 -- GlaxoSmithKline  
 Granulocyte colony-stimulating factor --  
     Dong-A  
 Granulocyte colony-stimulating factor  
     conjugate  
 grass allergy therapy -- Dynavax  
 GRF1-44 -- ICN  
 Growth Factor -- Chiron, Atrigel, Atrix,  
     Innogenetics, ZymoGenetics, Novo  
 growth factor peptides -- Biotherapeutics  
 growth hormone -- LG Chem  
 growth hormone, Recombinant human --  
     Serono  
 GT 4086 -- Gliatech  
 GW 353430 -- GlaxoSmithKline  
 GW-278884 -- GlaxoSmithKline  
 H 11 -- Viventia Biotech

H5N1 influenza A virus vaccine -- Protein  
     Sciences  
 haemoglobin -- Biopure  
 haemoglobin 3011, Recombinant -- Baxter  
     Healthcare  
 haemoglobin crosfumaril -- Baxter Intl.  
 haemoglobin stabilized -- Ajinomoto  
 haemoglobin, recombinant -- Apex  
 HAF -- Immune Response  
 Hantavirus vaccine  
 HB 19  
 HBNF -- Regeneron  
 HCC-1 -- Pharis  
 hCG -- Milkhaus  
 hCG vaccine -- Zonagen  
 HE-317 -- Hollis-Eden Pharmaceuticals  
 Heat shock protein cancer and influenza  
     vaccines -- StressGen  
 Helicobacter pylori vaccine -- Acambis,  
     AstraZeneca/CSL, Chiron, Provalis  
 Helistat-G -- GalaGen  
 Hemolink -- Hemosol  
 hepatpoietin -- Snow Brand  
 heparanase -- InSight  
 heparinase I -- Ibex  
 heparinase III -- Ibex  
 Hepatitis A vaccine -- American Biogenetic  
     Sciences  
 Hepatitis A vaccine inactivated  
 Hepatitis A vaccine Nothav -- Chiron  
 Hepatitis A-hepatitis B vaccine --  
     GlaxoSmithKline  
 hepatitis B therapy -- Tripep  
 Hepatitis B vaccine -- Amgen, Chiron SpA,  
     Meiji Milk, NIS, Prodeva, PowderJect,  
     Rhein Biotech  
 Hepatitis B vaccine recombinant -- Evans  
     Vaccines, Epitec Combiotech, Genentech,  
     MedImmune, Merck Sharp & Dohme,  
     Rhein Biotech, Shantha Biotechnics,  
     Vector, Yeda

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| Hepatitis B vaccine recombinant TGP 943 -- HIV peptides -- American Home Products   |  |
| Takeda  | HIV vaccine -- Applied bioTech., Axis  |
| Hepatitis C vaccine -- Bavarian Nordic, Chiron, Innogenetics Acambis,   | Genetics, Biogen, Bristol-Myers Squibb, Genentech, Korea Green Cross, NIS,   |
| Hepatitis D vaccine -- Chiron Vaccines  | Oncogen, Protein Sciences Corporation,   |
| Hepatitis E vaccine recombinant -- Genelabs/GlaxoSmithKline, Novavax  | Terumo, Tonen Corporation, Wyeth-Ayerst, Wyeth-Lederle Vaccines-Malvern,   |
| hepatocyte growth factor -- Panorama, Sosei   | Advanced BioScience Laboratories, Bavarian Nordic, Bavarian Nordic/Statens Serum Institute, GeneCure, Immune Response, Progenics, Therion Biologics, United Biomedical, Chiron |
| hepatocyte growth factor kringle fragments - - EntreMed   |  |
| Her-2/Neu peptides -- Corixa  |  |
| Herpes simplex glycoprotein DNA vaccine -- Merck, Wyeth-Lederle Vaccines-Malvern, Genentech, GlaxoSmithKline, Chiron, Takeda                              | HIV vaccine vCP1433 -- Aventis Pasteur   |
| Herpes simplex vaccine -- Cantab Pharmaceuticals, CEL-SCI, Henderson Morley   | HIV vaccine vCP1452 -- Aventis Pasteur   |
| Herpes simplex vaccine live -- ImClone Systems/Wyeth-Lederle, Aventis Pasteur   | HIV vaccine vCP205 -- Aventis Pasteur  |
| HGF derivatives -- Dompe  | HL-9 -- American BioScience  |
| hAPP vaccine -- Crucell   | HM-9239 -- Cytran  |
| Hib-hepatitis B vaccine -- Aventis Pasteur  | HML-103 -- Hemosol   |
| HIC 1   | HML-104 -- Hemosol   |
| HIP-- Altachem  | HML-105 -- Hemosol   |
| Hirudins -- Biopharma, Cangene, Dongkook, Japan Energy Corporation, Pharmacia Corporation, SIR International, Sanofi-Synthelabo, Sotragene, Rhein Biotech | HML-109 -- Hemosol   |
| HIV edible vaccine -- ProdiGene   | HML-110 -- Hemosol   |
| HIV gp120 vaccine -- Chiron, Ajinomoto, GlaxoSmithKline, ID Vaccine, Progenics, VaxGen  | HML-121 -- Hemosol   |
| HIV gp120 vaccine gene therapy --   | hNLP -- Pharis   |
| HIV gp160 DNA vaccine -- PowderJect, Aventis Pasteur, Oncogen, Hyland Immuno, Protein Sciences  | Hookworm vaccine   |
| HIV gp41 vaccine -- Panacos   | host-vector vaccines -- Henogen  |
| HIV HGP-30W vaccine -- CEL-SCI  | HPM 1 -- Chugai  |
| HIV immune globulin -- Abbott, Chiron   | HPV vaccine -- MediGene  |
|   | HSA -- Meristem  |
|   | HSF -- StressGen   |
|   | HSP carriers -- Weizmann, Yeda, Peptor   |
|   | HSPPC-70 -- Antigenics   |
|   | HSPPC-96 -- pathogen-derived -- Antigenics   |
|   | HSV 863 -- Novartis  |
|   | HTLV-I DNA vaccine   |
|   | HTLV-I vaccine   |
|   | HTLV-II vaccine -- Access  |
|   | HU 901 -- Tanox  |
|   | Hu23F2G -- ICOS  |
|   | HuHMFG1  |

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|--|---|
| HumaLYM -- Intracell                       | HuMax-IL15 -- Genmab                                |
| Human krebs statika -- Yamanouchi          | HYB 190 -- Hybridon                                 |
| human monoclonal antibodies --             | HYB 676 -- Hybridon                                 |
| Abgenix/Biogen, Abgenix/ Corixa,           | I-125 MAb A33 -- Celltech                           |
| Abgenix/Immunex, Abgenix/Lexicon,          | Ibritumomab tiuxetan -- IDEC                        |
| Abgenix/ Pfizer, Athersys/Medarex,         | IBT-9401 -- Ibex                                    |
| Biogen/MorphoSys, CAT/Searle,              | IBT-9402 -- Ibex                                    |
| Centocor/Medarex, Corixa/Kirin Brewery,    | IC 14 -- ICOS                                       |
| Corixa/Medarex, Eos BioTech./Medarex,      | Idarubicin anti-Ly-2.1 --                           |
| Eos/Xenerex, Exelixis/Protein Design       | IDEc 114 -- IDEC                                    |
| Labs, ImmunoGen/ Raven,                    | IDEc 131 -- IDEC                                    |
| Medarex/B.Twelve,                          | IDEc 152 -- IDEC                                    |
| MorphoSys/ImmunoGen, XTL                   | IDM 1 -- IDM  |
| Blopharmaceuticals/Dyax,                   | IDPS -- Hollis-Eden Pharmaceuticals                 |
| Human monoclonal antibodies --             | iduronate-2-sulfatase -- Transkaryotic              |
| Medarex/Northwest Biotherapeutics,         | Therapies   |
| Medarex/Seattle Genetics                   | IGF/IBP-2-13 -- Pharis                              |
| human netrin-1 -- Exelixis                 | IGN-101 -- Igeneon                                  |
| human papillomavirus antibodies -- Epicyte | IK HIR02 -- Iketon                                  |
| Human papillomavirus vaccine -- Biotech    | IL-11 -- Genetics Institute/AHP                     |
| Australia, IDEC, StressGen                 | IL-13-PE38 -- NeoPharm                              |
| Human papillomavirus vaccine MEDI 501 --   | IL-17 receptor -- Immunex                           |
| MedImmune/GlaxoSmithKline                  | IL-18BP -- Yeda                                     |
| Human papillomavirus vaccine MEDI          | IL-1Hy1 -- Hyseq                                    |
| 503/MEDI 504 --                            | IL-1 $\beta$ -- Celltech                            |
| MedImmune/GlaxoSmithKline                  | IL-1 $\beta$ adjuvant -- Celltech                   |
| Human papillomavirus vaccine TA-CIN --     | IL-2 -- Chiron                                      |
| Cantab Pharmaceuticals                     | IL-2 + IL-12 -- Hoffman La-Roche                    |
| Human papillomavirus vaccine TA-HPV --     | IL-6/sIL-6R fusion -- Hadasit                       |
| Cantab Pharmaceuticals                     | IL-6R derivative -- Tosoh                           |
| Human papillomavirus vaccine TH-GW --      | IL-7-Dap 389 fusion toxin -- Ligand                 |
| Cantab/GlaxoSmithKline                     | IM-862 -- Cytran                                    |
| human polyclonal antibodies -- Biosite/Eos | IMC-1C11 -- ImClone                                 |
| BioTech./ Medarex                          | imiglucerase -- Genzyme                             |
| human type II anti factor VIII monoclonal  | immune globulin intravenous (human) --              |
| antibodies -- ThromboGenics                | Hoffman La Roche                                    |
| humanised anti glycoprotein Ib murine      | immune privilege factor -- Proneuron                |
| monoclonal antibodies -- ThromboGenics     | Immunocal -- Immunotec                              |
| HumaRAD -- Intracell                       | Immunogene therapy -- Briana Bio-Tech               |
| HuMax EGFR -- Genmab                       | Immunoliposomal 5-fluorodeoxyuridine-dipalmitate -- |
| HuMax-CD4 -- Medarex                       |   |

immunosuppressant vaccine -- Aixlie  
 immunotoxin -- Antisoma, NIH  
 ImmuRAIT-Re-188 -- Immunomedics  
 imreg-1 -- Imreg  
 infertility -- Johnson & Johnson, E-TRANS  
 Influenza virus vaccine -- Aventis Pasteur,  
     Protein Sciences  
 inhibin -- Biotech Australia, Human  
     Therapeutics  
 Inhibitory G protein gene therapy  
 INKP-2001 -- InKine  
 Inolimomab -- Diaclone  
 insulin -- AutoImmune, Altea, Biobras,  
     BioSante, Bio-Tech. General, Chong Kun  
     Dang, Emisphere, Flamel, Provalis, Rhein  
     Biotech, TranXenoGen  
 insulin (bovine) -- Novartis  
 insulin analogue -- Eli Lilly  
 Insulin Aspart -- Novo Nordisk  
 insulin detemir -- Novo Nordisk  
 insulin glargine -- Aventis  
 insulin inhaled -- Inhale Therapeutics  
     Systems, Alkermes  
 insulin oral -- Inovax  
 insulin, AeroDose -- AeroGen  
 insulin, AERx -- Aradigm  
 insulin, BEODAS -- Elan  
 insulin, Biphasix -- Helix  
 insulin, buccal -- Generex  
 insulin, I2R -- Flemington  
 insulin, intranasal -- Bentley  
 insulin, oral -- Nobex, Unigene  
 insulin, Orasome -- Endorex  
 insulin, ProMaxx -- Epic  
 insulin, Quadrant -- Elan  
 insulin, recombinant -- Aventis  
 insulin, Spiros -- Elan  
 insulin, Transfersome -- IDEA  
 insulin, Zymo, recombinant -- Novo Nordisk  
 insulinotropin -- Scios  
 Insulysin gene therapy --  
     integrin antagonists -- Merck  
     interferon (Alpha2) -- SRC VB VECTOR,  
         Viragen, Dong-A, Hoffman La-Roche,  
         Genentech  
     interferon -- BioMedicines, Human Genome  
         Sciences  
     interferon (Alfa-n3) -- Interferon Sciences  
         Intl.  
     interferon (Alpha), Biphasix -- Helix  
     interferon (Alpha) -- Amgen, BioNative,  
         Novartis, Genzyme Transgenics,  
         Hayashibara, Inhale Therapeutics  
         Systems, Medusa, Flamel, Dong-A,  
         GeneTrol, Nastech, Shantha,  
         Wassermann, LG Chem, Sumitomo,  
         Aventis, Behring EGIS, Pepgen, Servier,  
         Rhein Biotech,  
     interferon (Alpha2A)  
     interferon (Alpha2B) -- Enzon, Schering-  
         Plough, Biogen, IDEA  
     interferon (Alpha-N1) -- GlaxoSmithKline  
     interferon (beta) -- Rentschler, GeneTrol,  
         Meristem, Rhein Biotech, Toray, Yeda,  
         Daichi, Mochida  
     interferon (Beta1A) -- Serono, Biogen  
     interferon (beta1A), inhale -- Biogen  
     interferon ( $\beta$ 1b) -- Chiron  
     interferon (tau) -- Pepgen  
     Interferon alfacon-1 -- Amgen  
     Interferon alpha-2a vaccine  
     Interferon Beta 1b -- Schering/Chiron,  
         InterMune  
     Interferon Gamma -- Boehringer Ingelheim,  
         Sheffield, Rentschler, Hayashibara  
     interferon receptor, Type I -- Serono  
     interferon(Gamma1B) -- Genentech  
     Interferon-alpha-2b + ribavirin -- Biogen,  
         ICN  
     Interferon-alpha-2b gene therapy --  
         Schering-Plough  
     Interferon-con1 gene therapy --

interleukin-1 antagonists -- Dompe  
 Interleukin-1 receptor antagonist -- Abbott  
     Bioresearch, Pharmacia  
 Interleukin-1 receptor type I -- Immunex  
 interleukin-1 receptor Type II -- Immunex  
 Interleukin-10 -- DNAX, Schering-Plough  
 Interleukin-10 gene therapy --  
 interleukin-12 -- Genetics Institute, Hoffman La-Roche  
     Sanofi  
 interleukin-13 antagonists -- AMRAD  
 Interleukin-13-PE38QQR  
 interleukin-15 -- Immunex  
 interleukin-16 -- Research Corp  
 interleukin-18 -- GlaxoSmithKline  
 Interleukin-1-alpha -- Immunex/Roche  
 interleukin-2 -- SRC VB VECTOR,  
     Ajinomoto, Biomira  
 Interleukin-3 -- Cangene  
 Interleukin-4 -- Immunology Ventures,  
     Sanofi Winthrop, Schering-Plough,  
     Immunex/ Sanofi Winthrop, Bayer, Ono  
 interleukin-4 + TNF-Alpha -- NIH  
 interleukin-4 agonist -- Bayer  
 interleukin-4 fusion toxin -- Ligand  
 Interleukin-4 receptor -- Immunex, Immun  
 Interleukin-6 -- Ajinomoto, Cangene, Yeda,  
     Genetics Institute, Novartis  
 interleukin-6 fusion protein --  
 interleukin-6 fusion toxin -- Ligand, Serono  
 interleukin-7 -- IC Innovations  
 interleukin-7 receptor -- Immunex  
 interleukin-8 antagonists -- Kyowa  
     Hakko/Millennium/Pfizer  
 interleukin-9 antagonists -- Genaera  
 interleukins -- Cel-Sci  
 Iodine I 131 tositumomab -- Corixa  
 lor EPOCIM -- Center of Molecular  
     Immunology  
 lor-P3 -- Center of Molecular Immunology  
 IP-10 -- NIH  
     IPF -- Metabolex  
     IR-501 -- Immune Response  
     ISIS 9125 -- Isis Pharmaceuticals  
     ISURF No. 1554 -- Millennium  
     ISURF No. 1866 -- Iowa State Univer.  
     ITF-1697 -- Italfarmaco  
     IxC 162 -- Ixion  
     J 695 -- Cambridge Antibody Tech.,  
         Genetics Inst., Knoll  
     Jagged + FGF -- Repair  
     JKC-362 -- Phoenix Pharmaceuticals  
     JTP-2942 -- Japan Tobacce  
     Juman monoclonal antibodies --  
         Medarex/Raven  
     K02 -- Axys Pharmaceuticals  
     Keliximab -- IDEC  
     Keyhole limpet haemocyanin  
     KGF -- Amgen  
     KM 871 -- Kyowa  
     KPI 135 -- Scios  
     KPI-022 -- Scios  
     Kringle 5  
     KSB 304  
     KSB-201 -- KS Biomedix  
     L 696418 -- Merck  
     L 703801 -- Merck  
     L1 -- Acorda  
     L-761191 -- Merck  
     lactoferrin -- Meristem, Pharming, Agennix  
     lactoferrin cardio -- Pharming  
     LAG-3 -- Serono  
     LAIT -- GEMMA  
     LAK cell cytotoxin -- Arizona  
     lamellarins -- PharmaMar/University of  
         Malaga  
     laminin A peptides -- NIH  
     lanoteplase -- Genetics Institute  
     laronidase -- BioMarin  
     Lassa fever vaccine  
     LCAT -- NIH  
     LDP 01 -- Millennium

LDP 02 -- Millennium  
 Lecithinized superoxide dismutase -- Seikagaku  
 LeIF adjuvant -- Corixa  
 leishmaniasis vaccine -- Corixa  
 lenercept -- Hoffman La-Roche  
 Lenograstim -- Aventis, Chugai  
 lepirudin -- Aventis  
 leptin -- Amgen, IC Innovations  
 Leptin gene therapy -- Chiron Corporation  
 leptin, 2nd-generation -- Amgen  
 leridistim -- Pharmacia  
 leuprolide, ProMaxx -- Epic  
 leuprorelin, oral -- Unigene  
 LeuTech -- Papatin  
 LEX 032 -- SuperGen  
 LiDEPT -- Novartis  
 lipase -- Altus Biologics  
 lipid A vaccine -- EntreMed  
 lipid-linked anchor Tech. -- ICRT, ID Biomedical  
 liposome-CD4 Tech. -- Sheffield  
 Listeria monocytogenes vaccine  
 LMB 1  
 LMB 7  
 LMB 9 -- Battelle Memorial Institute, NIH  
 LM-CD45 -- Cantab Pharmaceuticals  
 lovastatin -- Merck  
 LSA-3  
 LT- $\beta$  receptor -- Biogen  
 lung cancer vaccine -- Corixa  
 lusupultide -- Scios  
 L-Vax -- AVAX  
 LY 355455 -- Eli Lilly  
 LY 366405 -- Eli Lilly  
 LY-355101 -- Eli Lilly  
 Lyme disease DNA vaccine -- Vical/Aventis  
 Pasteur  
 Lyme disease vaccine -- Aquila  
 Biopharmaceuticals, Aventis, Pasteur,  
 Symbicom, GlaxoSmithKline, Hyland  
 Immuno, MedImmune  
 Lymphocytic choriomeningitis virus vaccine  
 lymphoma vaccine -- Biomira, Genotope  
 LYP18  
 lys plasminogen, recombinant  
 Lysosomal storage disease gene therapy -- Avigen  
 lysostaphin -- Nutrition 21  
 M 23 -- Gruenthal  
 M1 monoclonal antibodies -- Acorda Therapeutics  
 MA 16N7C2 -- Corvas Intl.  
 malaria vaccine -- GlaxoSmithKline,  
 AdProTech, Antigenics, Apovia, Aventis  
 Pasteur, Axis Genetics, Behringwerke,  
 CDCP, Chiron Vaccines, Genzyme  
 Transgenics, Hawaii, MedImmune, NIH,  
 NYU, Oxxon, Roche/Saramane, Biotech Australia, Rx Tech  
 Malaria vaccine CDC/NIIMALVAC-1  
 malaria vaccine, multicomponent  
 gammaglobulin -- Corixa  
 mammastatin -- Biotherapeutics  
 mannan-binding lectin -- NatlImm  
 mannan-MUC1 -- Psiron  
 MAP 30  
 Marinovir -- Phytera  
 MARstem -- Maret  
 MB-015 -- Mochida  
 MBP -- ImmuLogic  
 MCI-028 -- Mitsubishi-Tokyo  
 MCIF -- Human Genome Sciences  
 MDC -- Advanced BioScience -- Akzo Nobel, ICOS  
 MDX 11 -- Medarex  
 MDX 210 -- Medarex  
 MDX 22 -- Medarex  
 MDX 22

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| MDX 240 -- Medarex                        | Methionine lyase gene therapy --             |
| MDX 33                                    | AntiCancer                                   |
| MDX 44 -- Medarex                         | Met-RANTES -- Genexa Biomedical,             |
| MDX 447 -- Medarex                        | Serono                                       |
| MDX H210 -- Medarex                       | Metreleptin                                  |
| MDX RA -- Houston BioTech., Medarex       | MGDF -- Kirin                                |
| ME-104 -- Pharmexa                        | MGV -- Progenics                             |
| Measles vaccine                           | micrin -- Endocrine                          |
| Mecasermin -- Cephalon/Chiron, Chiron     | microplasmin -- ThromboGenics                |
| MEDI 488 -- MedImmune                     | MIF -- Genetics Institute                    |
| MEDI 500                                  | migration inhibitory factor -- NIH           |
| MEDI 507 -- BioTransplant                 | Mim CD4.1 -- Xycte Therapies                 |
| melanin concentrating hormone --          | mirostipen -- Human Genome Sciences          |
| Neurocrine Biosciences                    | MK 852 -- Merck                              |
| melanocortins -- OMRF                     | Mobenakin -- NIS                             |
| Melanoma monoclonal antibodies -- Viragen | molgramostim -- Genetics Institute, Novartis |
| melanoma vaccine -- GlaxoSmithKline,      | monoclonal antibodies -- Abgenix/Celltech,   |
| Akzo Nobel, Avant, Aventis Pasteur,       | Immusol/ Medarex, Viragen/ Roslin            |
| Bavarian Nordic, Biovector, CancerVax,    | Institute, Cambridge Antibody Tech./Elan     |
| Genzyme Molecular Oncology, Humbolt,      | MAb 108 --                                   |
| ImClone Systems, Memorial, NYU, Oxxon     | MAb 10D5 --                                  |
| Melanoma vaccine Magevac -- Therion       | MAb 14.18-interleukin-2 immunocytokine --    |
| memory enhancers -- Scios                 | Lexigen                                      |
| meningococcal B vaccine -- Chiron         | MAb 14G2a --                                 |
| meningococcal vaccine -- CAMR             | MAb 15A10 --                                 |
| Meningococcal vaccine group B conjugate - | MAb 170 -- Biomira                           |
| - North American Vaccine                  | MAb 177Lu CC49 --                            |
| Meningococcal vaccine group B             | MAb 17F9                                     |
| recombinant -- BioChem Vaccines,          | MAb 1D7                                      |
| Microscience                              | MAb 1F7 -- Immune Network                    |
| Meningococcal vaccine group Y conjugate - | MAb 1H10-doxorubicin conjugate               |
| - North American Vaccine                  | MAb 26-2F                                    |
| Meningococcal vaccine groups A B and C    | MAb 2A11                                     |
| conjugate -- North American Vaccine       | MAb 2E1 -- RW Johnson                        |
| Mepolizumab -- GlaxoSmithKline            | MAb 2F5                                      |
| Metastatin -- EntreMed, Takeda            | MAb 31.1 -- International BioImmune          |
| Met-CkB7 -- Human Genome Sciences         | Systems                                      |
| met-enkephalin -- TNI                     | MAb 32 -- Cambridge Antibody Tech.,          |
| METH-1 -- Human Genome Sciences           | Peptech                                      |
| methioninase -- AntiCancer                | MAb 323A3 -- Centocor                        |
|   | MAb 3C5                                      |

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| MAb 3F12   | MAb C242-PE conjugate                           |
| MAb 3F8  | MAb c30-6                                       |
| MAb 42/6   | MAb CA208-cytorhodin-S conjugate --             |
| MAb 425 -- Merck KGaA  | Hoechst Japan                                   |
| MAb 447-52D -- Merck Sharp & Dohme                                 | MAb CC49 -- Enzon                               |
| MAb 45-2D9- -- haematoporphyrin<br>conjugate                       | MAb ch14.18 --                                  |
| MAb 4B4  | MAb CH14.18-GM-CSF fusion protein --<br>Lexigen |
| MAb 4E3-CPA conjugate -- BCM Oncologia                             | MAb chCE7                                       |
| MAb 4E3-daunorubicin conjugate                                     | MAb CI-137 -- AMRAD                             |
| MAb 50-6   | MAb cisplatin conjugate                         |
| MAb 50-61A – Institut Pasteur                                      | MAb CLB-CD19                                    |
| MAb 5A8 -- Biogen  | MAb CLB-CD19v                                   |
| MAb 791T/36-methotrexate conjugate                                 | MAb CLL-1 -- Peregrine                          |
| MAb 7c11.e8  | MAb CLL-1-GM-CSF conjugate                      |
| MAb 7E11 C5-selenocystamine conjugate                              | MAb CLL-1-IL-2 conjugate -- Peregrine           |
| MAb 93KA9 -- Novartis  | MAb CLN IgG -- doxorubicin conjugates           |
| MAb A5B7-cisplatin conjugate --<br>Biodynamics Research, Pharmacia | MAb conjugates – Tanox                          |
| MAb A5B7-I-131   | MAb D612  |
| MAb A7   | MAb Dal B02                                     |
| MAb A717 -- Exocell  | MAb DC101 -- ImClone                            |
| MAb A7-zinostatin conjugate  | MAb EA 1 –                                      |
| MAb ABX-RB2 -- Abgenix   | MAb EC708 -- Biovation                          |
| MAb ACA 11   | MAb EP-5C7 -- Protein Design Labs               |
| MAb AFP-I-131 – Immunomedics                                       | MAb ERIC-1 -- ICRT                              |
| MAb AP1  | MAb F105 gene therapy                           |
| MAb AZ1  | MAb FC 2.15                                     |
| MAb B3-LysPE40 conjugate   | MAb G250 -- Centocor                            |
| MAb B4 – United Biomedical   | MAb GA6   |
| MAb B43 Genistein-conjugate  | MAb GA733                                       |
| MAb B43.13-Tc-99m -- Biomira                                       | MAb Gliomab-H -- Viventia Biotech               |
| MAb B43-PAP conjugate  | MAb HB2-saporin conjugate                       |
| MAb B4G7-gelonin conjugate   | MAb HD 37 –                                     |
| MAb BCM 43-daunorubicin conjugate --<br>BCM Oncologia              | MAb HD37-ricin chain-A conjugate                |
| MAb BIS-1  | MAb HNK20 -- Acambis                            |
| MAb BMS 181170 -- Bristol-Myers Squibb                             | MAb huN901-DM1 conjugate --<br>ImmunoGen        |
| MAb BR55-2   | MAb I-131 CC49 -- Corixa                        |
| MAb BW494  | MAb ICO25                                       |
| MAb C 242-DM1 conjugate -- ImmunoGen                               | MAb ICR12-CPG2 conjugate                        |
|  | MAb ICR-62                                      |

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| MAb IRac-ricin A conjugate                               | MAb R-24   |
| MAb K1   | MAb R-24 α Human GD3 -- Celltech                     |
| MAb KS1-4-methotrexate conjugate                         | MAb RFB4-ricin chain A conjugate                     |
| MAb L6 -- Bristol-Myers Squibb, Oncogen                  | MAb RFT5-ricin chain A conjugate                     |
| MAb LiCO 16-88   | MAb SC 1   |
| MAb LL2-I-131 – Immunomedics                             | MAb SM-3 -- ICRT                                     |
| MAb LL2-Y-90   | MAb SMART 1D10 -- Protein Design Labs                |
| MAb LS2D617 -- Hybritech                                 | MAb SMART ABL 364 -- Novartis                        |
| MAb LYM-1-gelonin conjugate                              | MAb SN6f   |
| MAb LYM-1-I-131  | MAb SN6f-deglycosylated ricin A chain<br>conjugate – |
| MAb LYM-1-Y-90   | MAb SN6j   |
| MAb LYM-2 -- Peregrine                                   | MAb SN7-ricin chain A conjugate                      |
| MAb M195   | MAb T101-Y-90 conjugate -- Hybritech                 |
| MAb M195-bismuth 213 conjugate --<br>Protein Design Labs | MAb T-88 -- Chiron                                   |
| MAb M195-gelonin conjugate                               | MAb TB94 -- Cancer ImmunoBiology                     |
| MAb M195-I-131   | MAb TEC 11   |
| MAb M195-Y-90  | MAb TES-23 -- Chugai                                 |
| MAb MA 33H1 -- Sanofi                                    | MAb TM31 -- Avant                                    |
| MAb MAD11  | MAb TNT-1 -- Cambridge Antibody Tech.,<br>Peregrine  |
| MAb MGb2   | MAb TNT-3  |
| MAb MINT5  | MAb TNT-3 -- IL2 fusion protein –                    |
| MAb MK2-23   | MAb TP3-At-211                                       |
| MAb MOC31 ETA(252-613) conjugate                         | MAb TP3-PAP conjugate –                              |
| MAb MOC-31-In-111  | MAb UJ13A -- ICRT                                    |
| MAb MOC-31-PE conjugate                                  | MAb UN3  |
| MAb MR6 –  | MAb ZME-018-gelonin conjugate                        |
| MAb MRK-16 -- Aventis Pasteur                            | MAb-BC2 -- GlaxoSmithKline                           |
| MAb MS11G6   | MAb-DM1 conjugate -- ImmunoGen                       |
| MAb MX-DTPA BrE-3  | MAb-ricin-chain-A conjugate -- XOMA                  |
| MAb MY9  | MAb-temoporfin conjugates                            |
| MAb Nd2 -- Tosoh   | Monopharm C -- Viventia Biotech                      |
| MAb NG-1 -- Hygeia                                       | monteplase -- Eisai                                  |
| MAb NM01 – Nissin Food                                   | montirelin hydrate -- Gruenenthal                    |
| MAb OC 125   | moroctocog alfa -- Genetics Institute                |
| MAb OC 125-CMA conjugate                                 | Moroctocog-alfa -- Pharmacia                         |
| MAb OKI-1 -- Ortho-McNeil                                | MP 4   |
| MAb OX52 -- Bioproducts for Science                      | MP-121 -- Biopharm                                   |
| MAb PMA5   | MP-52 -- Biopharm                                    |
| MAb PR1  | MRA -- Chugai  |
| MAb prost 30   |  |

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|--|---|
| MS 28168 -- Mitsui Chemicals, Nihon Schering                   | Neuroprotective vaccine -- University of Auckland |
| MSH fusion toxin -- Ligand                                     | neurotrophic chimaeras -- Regeneron               |
| MSI-99 -- Genaera  | neurotrophic factor -- NsGene, CereMedix          |
| MT 201 -- Micromet   | NeuroVax -- Immune Response                       |
| Muc-1 vaccine -- Corixa  | neurturin -- Genentech                            |
| mucosal tolerance -- Aberdeen                                  | neutral endopeptidase -- Genentech                |
| mullerian inhibiting subst                                     | NGF enhancers -- NeuroSearch                      |
| muplestim -- Genetics Institute, Novartis, DSM Anti-Infectives | NHL vaccine -- Large Scale Biology                |
| murine MAb -- KS Biomedix                                      | NIP45 -- Boston Life Sciences                     |
| Mutant somatropin -- JCR Pharmaceutical                        | NKI-B20   |
| MV 833 -- Toagosei   | NM 01 -- Nissin Food                              |
| Mycoplasma pulmonis vaccine                                    | NMI-139 -- NitroMed                               |
| Mycoprex -- XOMA   | NMMP -- Genetics Institute                        |
| myeloperoxidase -- Henogen                                     | NN-2211 -- Novo Nordisk                           |
| myostatin -- Genetics Institute                                | Noggin -- Regeneron                               |
| Nacolomab tafenatox -- Pharmacia                               | Nonacog alfa                                      |
| nagrestipen -- British Biotech                                 | Norelin -- Biostar                                |
| NAP-5 -- Corvas Intl.  | Norwalk virus vaccine                             |
| NAPc2 -- Corvas Intl.  | NRLU 10 -- NeoRx                                  |
| nartograstim -- Kyowa  | NRLU 10 PE -- NeoRx                               |
| Natalizumab -- Protein Design Labs                             | NT-3 -- Regeneron                                 |
| Nateplase -- NIH, Nihon Schering                               | NT-4/5 -- Genentech                               |
| nateplase -- Schering AG                                       | NU 3056   |
| NBI-3001 -- Neurocrine Biosci.                                 | NU 3076   |
| NBI-5788 -- Neurocrine Biosci.                                 | NX 1838 -- Gilead Sciences                        |
| NBI-6024 -- Neurocrine Biosci.                                 | NY ESO-1/CAG-3 antigen -- NIH                     |
| Nef inhibitors -- BRI  | NYVAC-7 -- Aventis Pasteur                        |
| Neisseria gonorrhoea vaccine -- Antex Biologics                | NZ-1002 -- Novazyme                               |
| Neomycin B-arginine conjugate                                  | obesity therapy -- Nobex                          |
| Nerelimomab -- Chiron  | OC 10426 -- Ontogen                               |
| Nerve growth factor -- Amgen -- Chiron, Genentech              | OC 144093 -- Ontogen                              |
| Nerve growth factor gene therapy                               | OCIF -- Sankyo                                    |
| nesiritide citrate -- Scios                                    | Oct-43 -- Otsuka                                  |
| neuregulin-2 -- CeNeS  | OK PSA - liposomal                                |
| neurocan -- NYU  | OKT3-gamma-1-ala-ala                              |
| neuronal delivery system -- CAMR                               | OM 991  |
|  | OM 992  |
|  | Omalizumab -- Genentech                           |
|  | oncoimmunin-L -- NIH                              |
|  | Oncolysin B -- ImmunoGen                          |

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| Oncolysin CD6 -- ImmunoGen                     | PAM 4 -- Merck   |
| Oncolysin M -- ImmunoGen                       | pamiteplase -- Yamanouchi  |
| Oncolysin S -- ImmunoGen                       | pancreatin, Minitabs -- Eurand   |
| Oncophage -- Antigenics                        | Pangen -- Fournier   |
| Oncostatin M -- Bristol-Myers Squibb           | Pantarin -- Selective Genetics   |
| OncoVax-CL -- Jenner Biotherapies              | Parainfluenza virus vaccine -- Pharmacia,  |
| OncoVax-P -- Jenner Biotherapies               | Pierre Fabre   |
| onercept -- Yeda                               | paraoxanase -- Esperion  |
| onychomycosis vaccine -- Boehringer Ingelheim  | parathyroid hormone -- Abiogen, Korea Green Cross  |
| opebecan -- XOMA                               | Parathyroid hormone (1-34) -- Chugai/Suntory   |
| opioids -- Arizona                             | Parkinson's disease gene therapy -- Cell Genesys/ Ceregene                                 |
| Orelvekin -- Genetics Institute                | Parvovirus vaccine -- MedImmune  |
| Org-33408 b-- Akzo Nobel                       | PCP-Scan -- Immunomedics   |
| Orolip DP -- EpiCept                           | PDGF cocktail -- Theratechnologies   |
| oryzacystatin                                  | peanut allergy therapy -- Dynavax  |
| OSA peptides -- GenSci Regeneration            | PEG anti-ICAM MAb -- Boehringer Ingelheim  |
| osteoblast-cadherin GF -- Pharis               | PEG asparaginase -- Enzon  |
| Osteocalcin-thymidine kinase gene therapy      | PEG glucocerebrosidase   |
| osteogenic protein -- Curis                    | PEG hirudin -- Knoll   |
| osteopontin -- OraPharma                       | PEG interferon-alpha-2a -- Roche   |
| osteoporosis peptides -- Integra, Telios       | PEG interferon-alpha-2b + ribavirin -- Biogen, Enzon, ICN Pharmaceuticals, Schering-Plough |
| osteoprotegerin -- Amgen, SnowBrand            | PEG MAb A5B7 --  |
| otitis media vaccines -- Antex Biologics       | Pegacaristim -- Amgen -- Kirin Brewery -- ZymoGenetics                                     |
| ovarian cancer -- University of Alabama        | Pegaldesleukin -- Research Corp  |
| OX40-IgG fusion protein -- Cantab, Xenova      | pegaspargase -- Enzon  |
| P 246 -- Diatide                               | pegfilgrastim -- Amgen   |
| P 30 -- Alfacell                               | PEG-interferon Alpha -- Viragen  |
| p1025 -- Active Biotech                        | PEG-interferon Alpha 2A -- Hoffman La-Roche  |
| P-113 <sup>A</sup> -- Demegen                  | PEG-interferon Alpha 2B -- Schering-Plough   |
| P-16 peptide -- Transition Therapeutics        | PEG-r-hirudin -- Abbott  |
| p43 -- Ramot                                   | PEG-uricase -- Mountain View   |
| P-50 peptide -- Transition Therapeutics        | Pegvisomant -- Genentech   |
| p53 + RAS vaccine -- NIH, NCI                  |  |
| PACAP(1-27) analogue                           |  |
| paediatric vaccines -- Chiron                  |  |
| Pafase -- ICOS                                 |  |
| PAGE-4 plasmid DNA -- IDEC                     |  |
| PAI-2 -- Biotech Australia, Human Therapeutics |  |
| Palivizumab -- MedImmune                       |  |

PEGylated proteins, PolyMASC -- Valentis Pharmaprojects No. 5947 -- StressGen  
 PEGylated recombinant native human leptin Pharmaprojects No. 5961 --  
 -- Roche  
 Pemtumomab  
 Penetratin -- Cyclacel  
 Pepscan – Antisoma  
 peptide G – Peptech, ICRT  
 peptide vaccine -- NIH ,NCI  
**Pexelizumab**  
 pexiganan acetate -- Genaera  
 Pharmaprojects No. 3179 -- NYU  
 Pharmaprojects No. 3390 -- Ernest Orlando  
 Pharmaprojects No. 3417 -- Sumitomo  
 Pharmaprojects No. 3777 -- Acambis  
 Pharmaprojects No. 4209 -- XOMA  
 Pharmaprojects No. 4349 – Baxter Intl.  
 Pharmaprojects No. 4651  
 Pharmaprojects No. 4915 -- Avanir  
 Pharmaprojects No. 5156 -- Rhizogenics  
 Pharmaprojects No. 5200 -- Pfizer  
 Pharmaprojects No. 5215 -- Origene  
 Pharmaprojects No. 5216 -- Origene  
 Pharmaprojects No. 5218 -- Origene  
 Pharmaprojects No. 5267 -- ML  
**Laboratories**  
 Pharmaprojects No. 5373 -- MorphoSys  
 Pharmaprojects No. 5493 -- Metabolex  
 Pharmaprojects No. 5707 -- Genentech  
 Pharmaprojects No. 5728 -- Autogen  
 Pharmaprojects No. 5733 -- BioMarin  
 Pharmaprojects No. 5757 -- NIH  
 Pharmaprojects No. 5765 -- Gryphon  
 Pharmaprojects No. 5830 -- AntiCancer  
 Pharmaprojects No. 5839 -- Dyax  
 Pharmaprojects No. 5849 -- Johnson & Johnson  
 Pharmaprojects No. 5860 -- Mitsubishi-Tokyo  
 Pharmaprojects No. 5869 – Oxford  
 GlycoSciences  
 Pharmaprojects No. 5883 -- Asahi Brewery  
 Theratechnologies  
 Pharmaprojects No. 5962 -- NIH  
 Pharmaprojects No. 5966 -- NIH  
 Pharmaprojects No. 5994 -- Pharming  
 Pharmaprojects No. 5995 -- Pharming  
 Pharmaprojects No. 6023 -- IMMUCON  
 Pharmaprojects No. 6063 -- Cytoclonal  
 Pharmaprojects No. 6073 -- SIDDCO  
 Pharmaprojects No. 6115 -- Genzyme  
 Pharmaprojects No. 6227 -- NIH  
 Pharmaprojects No. 6230 -- NIH  
 Pharmaprojects No. 6236 -- NIH  
 Pharmaprojects No. 6243 -- NIH  
 Pharmaprojects No. 6244 -- NIH  
 Pharmaprojects No. 6281 -- Senetek  
 Pharmaprojects No. 6365 -- NIH  
 Pharmaprojects No. 6368 -- NIH  
 Pharmaprojects No. 6373 -- NIH  
 Pharmaprojects No. 6408 – Pan Pacific  
 Pharmaprojects No. 6410 -- Athersys  
 Pharmaprojects No. 6421 – Oxford  
**GlycoSciences**  
 Pharmaprojects No. 6522 -- Maxygen  
 Pharmaprojects No. 6523 -- Pharis  
 Pharmaprojects No. 6538 -- Maxygen  
 Pharmaprojects No. 6554 -- APALEXO  
 Pharmaprojects No. 6560 -- Ardana  
 Pharmaprojects No. 6562 -- Bayer  
 Pharmaprojects No. 6569 -- Eos  
 Phenoxazine  
 Phenylase -- Ibex  
 Pigment epithelium derived factor – plasminogen activator inhibitor-1, recombinant -- DuPont Pharmaceuticals

Plasminogen activators -- Abbott Laboratories, American Home Products, Boehringer Mannheim, Chiron Corporation, DuPont Pharmaceuticals, Eli Lilly, Shionogi, Genentech, Genetics Institute, GlaxoSmithKline, Hemispherx Biopharma, Merck & Co, Novartis, Pharmacia Corporation, Wakamoto, Yeda  
 plasminogen-related peptides -- Bio-Tech. General/MGH  
 platelet factor 4 -- RepliGen  
 Platelet-derived growth factor -- Amgen -- ZymoGenetics  
 plusonermin -- Hayashibara  
 PMD-2850 -- Protherics  
 Pneumococcal vaccine -- Antex Biologics, Aventis Pasteur  
 Pneumococcal vaccine intranasal -- BioChem Vaccines/Biovector  
 PR1A3  
 PR-39  
 pralmorelin -- Kaken  
 Pretarget-Lymphoma -- NeoRx  
 Priliximab -- Centocor  
 PRO 140 -- Progenics  
 PRO 2000 -- Procept  
 PRO 367 -- Progenics  
 PRO 542 -- Progenics  
 pro-Apo A-I -- Esperion  
 prolactin -- Genzyme  
 Prosaptide TX14(A) -- Bio-Tech. General prostate cancer antibodies -- Immunex, UroCor  
 prostate cancer antibody therapy -- Genentech/UroGenesys, Genotherapeutics  
 prostate cancer immunotherapeutics -- The PSMA Development Company  
 prostate cancer vaccine -- Aventis Pasteur, Zonagen, Corixa, Dendreon, Jenner Biotherapies, Therion Biologics  
 prostate-specific antigen -- EntreMed  
 protein A -- RepliGen  
 protein adhesives -- Enzon  
 protein C -- Baxter Intl., PPL Therapeutics, ZymoGenetics  
 protein C activator -- Gilead Sciences  
 protein kinase R antags -- NIH  
 protirelin -- Takeda  
 protocadherin 2 -- Caprion  
 Pro-urokinase -- Abbott, Bristol-Myers Squibb, Dainippon, Tosoh -- Welfide  
 P-selectin glycoprotein ligand-1 -- Genetics Institute  
 pseudomonal infections -- InterMune  
 Pseudomonas vaccine -- Cytovax  
 PSGL-Ig -- American Home Products  
 PSP-94 -- Procyon  
 PTH 1-34 -- Nobex  
 Quilimmune-M -- Antigenics  
 R 101933  
 R 125224 -- Sankyo  
 RA therapy -- Cardion  
 Rabies vaccine recombinant -- Aventis Pasteur, BioChem Vaccines, Kaketsuken Pharmaceuticals  
 RadioTheraCIM -- YM BioSciences  
 Ramot project No. 1315 -- Ramot  
 Ramot project No. K-734A -- Ramot  
 Ramot project No. K-734B -- Ramot  
 RANK -- Immunex  
 ranpirnase -- Alfacell  
 ranpirnase-anti-CD22 MAb -- Alfacell  
 RANTES inhibitor -- Milan  
 RAPID drug delivery systems -- ARIAD  
 rasburicase -- Sanofi  
 rBPI-21, topical -- XOMA  
 RC 529 -- Corixa  
 rCFTR -- Genzyme Transgenics  
 RD 62198  
 rDnase -- Genentech  
 RDP-58 -- SangStat

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|---|---|
| RecepTox-Fce -- Keryx   | Ribozyme gene therapy -- Genset                 |
| RecepTox-GnRH -- Keryx, MTR Technologies  | Rickettsial vaccine recombinant                 |
| RecepTox-MBP -- Keryx, MTR Technologies   | RIGScan CR -- Neoprobe                          |
| recFSH -- Akzo Nobel, Organon   | RIP-3 -- Rigel                                  |
| REGA 3G12   | RK-0202 -- RxKinetix                            |
| Regavirumab -- Teijin   | RLT peptide -- Esperion                         |
| relaxin -- Connetics Corp   | rM/NEI -- IVAX                                  |
| Renal cancer vaccine -- Macropharm  | rmCRP -- Immtech                                |
| repifermin -- Human Genome Sciences   | RN-1001 -- Renovo                               |
| Respiratory syncytial virus PFP-2 vaccine -- Wyeth-Lederle                            | RN-3 -- Renovo                                  |
| Respiratory syncytial virus vaccine -- GlaxoSmithKline, Pharmacia, Pierre Fabre       | RNAse conjugate -- Immunomedics                 |
| Respiratory syncytial virus vaccine inactivated                                       | RO 631908 -- Roche                              |
| Respiratory syncytial virus-parainfluenza virus vaccine -- Aventis Pasteur, Pharmacia | Rotavirus vaccine -- Merck                      |
| Reteplase -- Boehringer Mannheim, Hoffman La-Roche                                    | RP 431 -- DuPont Pharmaceuticals                |
| Retropep -- Retroscreen   | RP-128 -- Resolution                            |
| RFB4 (dsFv) PE38  | RPE65 gene therapy --                           |
| RFI 641 -- American Home Products   | RPR 110173 -- Aventis Pasteur                   |
| RFTS -- UAB Research Foundation   | RPR 115135 -- Aventis Pasteur                   |
| RG 12986 -- Aventis Pasteur   | RPR 116258A -- Aventis Pasteur                  |
| RG 83852 -- Aventis Pasteur   | rPSGL-Ig -- American Home Products              |
| RG-1059 -- RepliGen   | r-SPC surfactant -- Byk Gulden                  |
| rGCR -- NIH   | rV-HER-2/neu -- Therion Biologics               |
| rGLP-1 -- Restoragen  | SA 1042 -- Sankyo                               |
| rGRF -- Restoragen  | sacrosidase -- Orphan Medical                   |
| rh Insulin -- Eli Lilly   | Sant 7  |
| RHAMM targeting peptides -- Cangene   | Sargramostim -- Immunex                         |
| rHb1.1 -- Baxter Intl.  | saruplase -- Gruenthal                          |
| rhCC10 -- Claragen  | Satumomab -- Cytogen                            |
| rhCG -- Serono  | SB 1 -- COR Therapeutics                        |
| Rheumatoid arthritis gene therapy   | SB 207448 -- GlaxoSmithKline                    |
| Rheumatoid arthritis vaccine -- Veterans Affairs Medical Center                       | SB 208651 -- GlaxoSmithKline                    |
| rhLH -- Serono  | SB 240683 -- GlaxoSmithKline                    |
|   | SB 249415 -- GlaxoSmithKline                    |
|   | SB 249417 -- GlaxoSmithKline                    |
|   | SB 6 -- COR Therapeutics                        |
|   | SB RA 31012 --                                  |
|   | SC 56929 -- Pharmacia                           |
|   | SCA binding proteins -- Curis, Enzon            |
|   | scFv(14E1)-ETA Berlex Laboratories, Schering AG |
|   | ScFv(FRP5)-ETA --                               |

ScFv6C6-PE40 --  
 SCH 55700 -- Celltech  
 Schistosomiasis vaccine -- Glaxo Wellcome/Medeva, Brazil  
 SCPF -- Advanced Tissue Sciences  
 scuPA-suPAR complex -- Hadasit  
 SD-9427 -- Pharmacia  
 SDF-1 -- Ono  
 SDZ 215918 -- Novartis  
 SDZ 280125 -- Novartis  
 SDZ 89104 -- Novartis  
 SDZ ABL 364 -- Novartis  
 SDZ MMA 383 -- Novartis  
 serine protease inhibs -- Pharis  
 sermorelin acetate -- Serono  
 SERP-1 -- Viron  
 sertenef -- Dainippon  
 serum albumin, Recombinant human -- Aventis Behring  
 serum-derived factor -- Hadasit  
 Sevirumab -- Novartis  
 SGN 14 -- Seattle Genetics  
 SGN 15 -- Seattle Genetics  
 SGN 17/19 -- Seattle Genetics  
 SGN 30 -- Seattle Genetics  
 SGN-10 -- Seattle Genetics  
 SGN-11 -- Seattle Genetics  
 SH 306 -- DuPont Pharmaceuticals  
 Shanvac-B -- Shantha  
 Shigella flexneri vaccine -- Avant, Acambis, Novavax  
 Shigella sonnei vaccine --  
 sICAM-1 -- Boehringer Ingelheim  
 Silteplase -- Genzyme  
 SIV vaccine -- Endocon, Institut Pasteur  
 SK 896 -- Sanwa Kagaku Kenkyusho  
 SK-827 -- Sanwa Kagaku Kenkyusho  
 Skeletex -- CellFactors  
 SKF 106160 -- GlaxoSmithKline  
 S-nitroso-AR545C --  
 SNTP -- Active Biotech  
 somatomedin-1 -- GroPep, Mitsubishi-Tokyo, NIH  
 somatomedin-1 carrier protein -- Insmed  
 somatostatin -- Ferring  
 Somatotropin/  
 Human Growth Hormone -- Bio-Tech. General, Eli Lilly  
 somatropin -- Bio-Tech. General, Alkermes, ProLease, Aventis Behring, Biovector, Cangene, Dong-A, Eli Lilly, Emisphere, Enact, Genentech, Genzyme Transgenics, Grandis/InfiMed, CSL, InfiMed, MacroMed, Novartis, Novo Nordisk, Pharmacia Serono, TranXenoGen  
 somatropin derivative -- Schering AG  
 somatropin, AIR -- Eli Lilly  
 Somatropin, inhaled -- Eli Lilly/Alkermes  
 somatropin, Kabi -- Pharmacia  
 somatropin, Orasome -- Novo Nordisk  
 Sonermin -- Dainippon Pharmaceutical  
 SP(V5.2)C -- Supertek  
 SPF66  
 sphingomyelinase -- Genzyme  
 SR 29001 -- Sanofi  
 SR 41476 -- Sanofi  
 SR-29001 -- Sanofi  
 SS1(dsFV)-PE38 -- NeoPharm  
 β2 microglobulin -- Avidex  
 β2-microglobulin fusion proteins -- NIH  
 β-amylid peptides -- CeNeS  
 β-defensin -- Pharis  
 Staphylococcus aureus infections -- Inhibitex/ZLB  
 Staphylococcus aureus vaccine conjugate -- Nabi  
 Staphylococcus therapy -- Tripep  
 Staphylokinase -- Biovation, Prothera, Thrombogenetics  
 Streptococcal A vaccine -- M6  
 Pharmaceuticals, North American Vaccine  
 Streptococcal B vaccine -- Microscience

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|---|---|
| Streptococcal B vaccine recombinant -- Biochem Vaccines               | TFPI -- EntreMed  |
| Streptococcus pyogenes vaccine  | tgD-IL-2 -- Takeda  |
| STRL-33 -- NIH  | TGF-Alpha -- ZymoGenetics                                       |
| Subalin -- SRC VB VECTOR  | TGF- $\beta$ -- Kolon   |
| SUIS -- United Biomedical   | TGF- $\beta$ 2 -- Insmed  |
| SUIS-LHRH -- United Biomedical  | TGF- $\beta$ 3 -- OSI   |
| SUN-E3001 -- Suntory  | Thalassaemia gene therapy -- Crucell                            |
| super high affinity monoclonal antibodies -- YM BioSciences           | TheraCIM-h-R3 -- Center of Molecular Immunology, YM BioSciences |
| Superoxide dismutase -- Chiron, Enzon, Ube Industries, Bio-Tech, Yeda | Theradigm-HBV -- Epimmune                                       |
| superoxide dismutase-2 -- OXIS  | Theradigm-HPV -- Epimmune                                       |
| suppressin -- UAB Research Foundation                                 | Theradigm-malaria -- Epimmune                                   |
| SY-161-P5 -- ThromboGenics  | Theradigm-melanoma -- Epimmune                                  |
| SY-162 -- ThromboGenics   | TheraFab -- Antisoma  |
| Systemic lupus erythematosus vaccine -- MedClone/VivoRx               | ThGRF 1-29 -- Theratechnologies                                 |
| T cell receptor peptide vaccine                                       | ThGRF 1-44 -- Theratechnologies                                 |
| T4N5 liposomes -- AGI Dermatics                                       | thrombomodulin -- Iowa, Novocastra                              |
| TACI, soluble -- ZymoGenetics   | Thrombopoietin -- Dragon Pharmaceuticals, Genentech             |
| targeted apoptosis -- Antisoma  | thrombopoietin, Pliva -- Receptron                              |
| tasonermin -- Boehringer Ingelheim                                    | Thrombospondin 2 --   |
| TASP  | thrombostatin -- Thromgen                                       |
| TASP-V  | thymalfasin -- SciClone   |
| Tat peptide analogues -- NIH  | thymocartin -- Gedeon Richter                                   |
| TBP I -- Yeda   | thymosin Alpha1 -- NIH  |
| TBP II  | thyroid stimulating hormone -- Genzyme                          |
| TBV25H -- NIH   | tICAM-1 -- Bayer  |
| Tc 99m for cea1 -- Center of Molecular Immunology                     | Tick anticoagulant peptide -- Merck                             |
| Tc 99m P 748 -- Diatide   | TIF -- Xoma   |
| Tc 99m votumumab -- Intracell   | Tifacogin -- Chiron, NIS, Pharmacia                             |
| Tc-99m rh-Annexin V -- Theseus Imaging                                | Tissue factor -- Genentech                                      |
| teceleukin -- Biogen  | Tissue factor pathway inhibitor                                 |
| tenecteplase -- Genentech   | TJN-135 -- Tsumura  |
| Teriparatide -- Armour Pharmaceuticals, Asahi Kasei, Eli Lilly        | TM 27 -- Avant  |
| terlipressin -- Ferring   | TM 29 -- Avant  |
| testisin -- AMRAD   | TMC-151 -- Tanabe Seiyaku                                       |
| Tetrafibrin -- Roche  | TNF tumour necrosis factor -- Asahi Kasei                       |
|   | TNF Alpha -- CytImmune  |
|   | TNF antibody -- Johnson & Johnson                               |
|   | TNF binding protein -- Amgen                                    |
|   | TNF degradation product -- Oncotech                             |

TNF receptor -- Immunex  
 TNF receptor 1, soluble -- Amgen  
 TNF Tumour necrosis factor-alpha -- Asahi Kasei, Genetech, Mochida  
 TNF-Alpha inhibitor -- Tripep  
 TNFRFc gene therapy -- Targeted Genetics  
 TNF-SAM2  
 ToleriMab -- Innogenetics  
 Toxoplasma gondii vaccine -- GlaxoSmithKline  
 TP 9201 -- Telios  
 TP10 -- Avant  
 TP20 -- Avant  
 tPA -- Centocor  
 trafermin -- Scios  
 TRAIL/Apo2L -- Immunex  
 transferrin-binding proteins -- CAMR  
 Transforming growth factor-beta-1 -- Genentech  
 transport protein -- Genesis  
 TRH -- Ferring  
 Triabin -- Schering AG  
 Triconal  
 Triflavin  
 troponin I -- Boston Life Sciences  
 TRP-2<sup>A</sup> -- NIH  
 trypsin inhibitor -- Mochida  
 TSP-1 gene therapy -- TT-232  
 TTS-CD2 -- Active Biotech  
 Tuberculosis vaccine -- Aventis Pasteur, Genesis  
 Tumor Targeted Superantigens -- Active Biotech -- Pharmacia  
 tumour vaccines -- PhotoCure  
 tumour-activated prodrug antibody conjugates -- Millennium/ImmunoGen  
 tumstatin -- ILEX  
 Tuvirumab -- Novartis  
 TV-4710 -- Teva  
 TWEAK receptor -- Immunex  
 TXU-PAP  
 TY-10721 -- TOA Eiyo  
 Type I diabetes vaccine -- Research Corp  
 Typhoid vaccine CVD 908  
 U 143677 -- Pharmacia  
 U 81749 -- Pharmacia  
 UA 1248 -- Arizona  
 UGIF -- Sheffield  
 UIC 2  
 UK 101  
 UK-279276 -- Corvas Intl.  
 urodilatin -- Pharis  
 urofollitrophin -- Serono  
 uteroferrin -- Pepgen  
 V 20 -- GLYCODEsign  
 V2 vasopressin receptor gene therapy vaccines -- Active Biotech  
 Varicella zoster glycoprotein vaccine -- Research Corporation Technologies  
 Varicella zoster virus vaccine live -- Cantab Pharmaceuticals  
 Vascular endothelial growth factor -- Genentech, University of California  
 Vascular endothelial growth factors -- R&D Systems  
 vascular targeting agents -- Peregrine  
 vasopermeation enhancement agents -- Peregrine  
 vasostatin -- NIH  
 VCL -- Bio-Tech. General  
 VEGF -- Genentech, Scios  
 VEGF inhibitor -- Chugai  
 VEGF-2 -- Human Genome Sciences  
 VEGF-Trap -- Regeneron  
 viscumin, recombinant -- Madaus  
 Vitaxin  
 Vitrase -- ISTA Pharmaceuticals  
 West Nile virus vaccine -- Bavarian Nordic  
 WP 652  
 WT1 vaccine -- Corixa  
 WX-293 -- Wilex BioTech.

WX-360 -- Wilex BioTech.  
WX-UK1 -- Wilex BioTech.  
XMP-500 -- XOMA  
XomaZyme-791 -- XOMA  
XTL 001 -- XTL Biopharmaceuticals  
XTL 002 -- XTL Biopharmaceuticals  
yeast delivery system -- Globelimmune  
Yersinia pestis vaccine  
YIGSR-Stealth -- Johnson & Johnson  
Yissum Project No. D-0460 -- Yissum

YM 207 -- Yamanouchi  
YM 337 -- Protein Design Labs  
Yttrium-90 labelled biotin  
Yttrium-90-labeled anti-CEA MAb T84.66 --  
ZD 0490 -- AstraZeneca  
ziconotide -- Elan  
ZK 157138 -- Berlex Laboratories  
Zolimomab aritox  
Zorcell -- Immune Response  
ZRXL peptides -- Novartis

| Protein  | Organism                      | EC#      | GenBank / GenPept                             |   | SwissProt        | PDB / 3D |
|--|-------------------------------|----------|---|---|------------------|----------|
| At1g08280  | <i>Arabidopsis thaliana</i>   | n.d.     | AC011438<br>BT004583<br>NC_003070             | AAF18241.1<br>AAO42829.1<br><b>NP_172305.1</b>                      | Q84W00<br>Q9SGD2 |          |
| At1g08660/F22O13.14  | <i>Arabidopsis thaliana</i>   | n.d.     | AC003981<br>AY064135<br>AY124807<br>NC_003070 | AAF99778.1<br><b>AAL36042.1</b><br>AAM70516.1<br><b>NP_172342.1</b> | Q8VZJ0<br>Q9FRR9 |          |
| At3g48820/T21J18_90  | <i>Arabidopsis thaliana</i>   | n.d.     | AY080589<br>AY133816<br>AL132963<br>NM_114741 | AAL85966.1<br>AAM91750.1<br><b>CAB87910.1</b><br>NP_190451.1        | Q8RY00<br>Q9M301 |          |
| $\alpha$ -2,3-sialyltransferase (ST3GAL-IV)                                    | <i>Bos taurus</i>             | n.d.     | AJ584673                                      | <b>CAE48298.1</b>   |                  |          |
| $\alpha$ -2,3-sialyltransferase (St3Gal-V)                                     | <i>Bos taurus</i>             | n.d.     | AJ585768                                      | <b>CAE51392.1</b>   |                  |          |
| $\alpha$ -2,6-sialyltransferase (Siat7b)                                       | <i>Bos taurus</i>             | n.d.     | AJ620651                                      | <b>CAF05850.1</b>   |                  |          |
| $\alpha$ -2,8-sialyltransferase (SIAT8A)                                       | <i>Bos taurus</i>             | 2.4.99.8 | AJ699418                                      | <b>CAG27880.1</b>   |                  |          |
| $\alpha$ -2,8-sialyltransferase (Siat8D)                                       | <i>Bos taurus</i>             | n.d.     | AJ699421                                      | <b>CAG27883.1</b>   |                  |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia-III (Siat8C)                            | <i>Bos taurus</i>             | n.d.     | AJ704563                                      | <b>CAG28696.1</b>   |                  |          |
| CMP $\alpha$ -2,6-sialyltransferase (ST6Gal I)                                 | <i>Bos taurus</i>             | 2.4.99.1 | Y15111<br>NM_177517                           | CAA75385.1<br>NP_803483.1   | O18974           |          |
| sialyltransferase 8 (fragment)   | <i>Bos taurus</i>             | n.d.     | AF450088                                      | <b>AAL47018.1</b>   | Q8WN13           |          |
| sialyltransferase ST3Gal-II (Siat4B)   | <i>Bos taurus</i>             | n.d.     | AJ748841                                      | <b>CAG44450.1</b>   |                  |          |
| sialyltransferase ST3Gal-III (Siat6)   | <i>Bos taurus</i>             | n.d.     | AJ748842                                      | <b>CAG44451.1</b>   |                  |          |
| sialyltransferase ST3Gal-VI (Siat10)   | <i>Bos taurus</i>             | n.d.     | AJ748843                                      | <b>CAG44452.1</b>   |                  |          |
| ST3Gal I   | <i>Bos taurus</i>             | n.d.     | AJ305086                                      | <b>CAC24698.1</b>   | Q9BEG4           |          |
| St6GalNAc-VI   | <i>Bos taurus</i>             | n.d.     | AJ620949                                      | <b>CAF06586.1</b>   |                  |          |
| CDS4   | <i>Branchiostoma floridae</i> | n.d.     | AF391289                                      | <b>AAM18873.1</b>   | Q8T771           |          |
| polysialyltransferase (PST) (fragment)<br>ST8Sia IV                            | <i>Cercopithecus aethiops</i> | 2.4.99.- | AF210729                                      | <b>AAF17105.1</b>   | Q9TT09           |          |
| polysialyltransferase (STX) (fragment)<br>ST8Sia II                            | <i>Cercopithecus aethiops</i> | 2.4.99.- | AF210318                                      | <b>AAF17104.1</b>   | Q9TT10           |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal I (Siat4)                               | <i>Ciona intestinalis</i>     | n.d.     | AJ626815                                      | <b>CAF25173.1</b>   |                  |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal I (Siat4)                               | <i>Ciona savignyi</i>         | n.d.     | AJ626814                                      | <b>CAF25172.1</b>   |                  |          |
| $\alpha$ -2,8-polysialyltransferase ST8Sia IV                                  | <i>Cricetulus griseus</i>     | 2.4.99.- | Z46801  | AAE28634<br>CAA86822.1  | <b>Q64690</b>    |          |
| Gal $\beta$ -1,3/4-GlcNAc $\alpha$ -2,3-sialyltransferase St3Gal I             | <i>Cricetulus griseus</i>     | n.d.     | AY266675                                      | <b>AAP22942.1</b>   | Q80WL0           |          |
| Gal $\beta$ -1,3/4-GlcNAc $\alpha$ -2,3-sialyltransferase St3Gal II (fragment) | <i>Cricetulus griseus</i>     | n.d.     | AY266676                                      | <b>AAP22943.1</b>   | Q80WK9           |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal I (Siat4)                               | <i>Danio rerio</i>            | n.d.     | AJ783740                                      | <b>CAH04017.1</b>   |                  |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal II (Siat5)                              | <i>Danio rerio</i>            | n.d.     | AJ783741                                      | <b>CAH04018.1</b>   |                  |          |

FIG. 2A

| Protein   | Organism                       | EC#      | GenBank / GenPept   |  | SwissProt        | PDB / 3D |
|---|--------------------------------|----------|---|--|------------------|----------|
| $\alpha$ -2,3-sialyltransferase ST3Gal III (Siat6)                    | <i>Danio rerio</i>             | n.d.     | AJ626821  | <b>CAF25179.1</b>  |                  |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal IV (Siat4c)                    | <i>Danio rerio</i>             | n.d.     | AJ744809  | <b>CAG32845.1</b>  |                  |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal V-r (Siat5-related)            | <i>Danio rerio</i>             | n.d.     | AJ783742  | <b>CAH04019.1</b>  |                  |          |
| $\alpha$ -2,6-sialyltransferase ST6Gal I (Siat1)                      | <i>Danio rerio</i>             | n.d.     | AJ744801  | <b>CAG32837.1</b>  |                  |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc II (Siat7B)                 | <i>Danio rerio</i>             | n.d.     | AJ634459  | <b>CAG25680.1</b>  |                  |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc V (Siat7E) (fragment)       | <i>Danio rerio</i>             | n.d.     | AJ646874  | <b>CAG26703.1</b>  |                  |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc VI (Siat7F) (fragment)      | <i>Danio rerio</i>             | n.d.     | AJ646883  | <b>CAG26712.1</b>  |                  |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia I (Siat 8A) (fragment)         | <i>Danio rerio</i>             | n.d.     | AJ715535  | <b>CAG29374.1</b>  |                  |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia III (Siat 8C) (fragment)       | <i>Danio rerio</i>             | n.d.     | AJ715543  | <b>CAG29382.1</b>  |                  |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia IV (Siat 8D) (fragment)        | <i>Danio rerio</i>             | n.d.     | AJ715545  | <b>CAG29384.1</b>  |                  |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia V (Siat 8E) (fragment)         | <i>Danio rerio</i>             | n.d.     | AJ715546  | <b>CAG29385.1</b>  |                  |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia VI (Siat 8F) (fragment)        | <i>Danio rerio</i>             | n.d.     | AJ715551  | <b>CAG29390.1</b>  |                  |          |
| $\beta$ -galactosamide $\alpha$ -2,6-sialyltransferase II (ST6Gal II) | <i>Danio rerio</i>             | n.d.     | AJ627627  | <b>CAF29495.1</b>  |                  |          |
| <i>N</i> -glycan $\alpha$ -2,8-sialyltransferase                      | <i>Danio rerio</i>             | n.d.     | BC050483<br>AY055462<br>NM_153662                         | AAH50483.1<br><b>AAL17875.1</b><br>NP_705948.1                             | Q7ZU51<br>Q8QH83 |          |
| ST3Gal III-related (siat6r)   | <i>Danio rerio</i>             | n.d.     | BC053179<br>AJ626820<br>NM_200355                         | AAH53179.1<br><b>CAF25178.1</b><br>NP_956649.1                             | Q7T3B9           |          |
| St3Gal-V  | <i>Danio rerio</i>             | n.d.     | AJ619960  | <b>CAF04061.1</b>  |                  |          |
| st6GalNAc-VI  | <i>Danio rerio</i>             | n.d.     | BC060932<br>AJ620947                                      | <b>AAH60932.1</b><br>CAF06584.1  |                  |          |
| $\alpha$ -2,6-sialyltransferase (CG4871) ST6Gal I                     | <i>Drosophila melanogaster</i> | 2.4.99.1 | AE003465<br>AF218237<br>AF397532<br>AE003465<br>NM_079129 | AAF47256.1<br><b>AAG13185.1</b><br>AAK92126.1<br>AAM70791.1<br>NP_523853.1 | Q9GU23<br>Q9W121 |          |
| $\alpha$ -2,3-sialyltransferase (ST3Gal-VI)                           | <i>Gallus gallus</i>           | n.d.     | AJ585767<br>AJ627204                                      | <b>CAE51391.1</b><br>CAF25503.1  |                  |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal I                              | <i>Gallus gallus</i>           | 2.4.99.4 | X80503<br>NM_205217                                       | <b>CAA56666.1</b><br>NP_990548.1   | Q11200           |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal IV (fragment)                  | <i>Gallus gallus</i>           | 2.4.99.- | AF035250  | AAC14163.1   | <b>O73724</b>    |          |
| $\alpha$ -2,3-sialyltransferase (ST3GAL-II)                           | <i>Gallus gallus</i>           | n.d.     | AJ585761  | <b>CAE51385.2</b>  |                  |          |
| $\alpha$ -2,6-sialyltransferase (Siat7b)                              | <i>Gallus gallus</i>           | n.d.     | AJ620653  | <b>CAF05852.1</b>  |                  |          |
| $\alpha$ -2,6-sialyltransferase ST6Gal I                              | <i>Gallus gallus</i>           | 2.4.99.1 | X755558<br>NM_205241                                      | <b>CAA53235.1</b><br>NP_990572.1   | Q92182           |          |
| $\alpha$ -2,6-sialyltransferase                                       | <i>Gallus gallus</i>           | 2.4.99.3 | -   | AAE68028.1   | Q92183           |          |

**FIG. 2B**

| Protein   | Organism |                      | EC#      | GenBank / GenPept  |  | SwissProt  | PDB / 3D |
|---|----------|----------------------|----------|--|--|--|----------|
| ST6GalNAc I   |          |                      |          | X74946<br>NM_205240  | AAE68029.1<br>CAA52902.1<br>NP_990571.1  |  |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc II                          |          | <i>Gallus gallus</i> | 2.4.99.- | X77775<br>NM_205233  | AAE68030.1<br>CAA54813.1<br>NP_990564.1  | Q92184   |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc III (SIAT7C) (fragment)     |          | <i>Gallus gallus</i> | n.d.     | AJ634455   | <b>CAG25677.1</b>  |  |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc V (SIAT7E) (fragment)       |          | <i>Gallus gallus</i> | n.d.     | AJ646877   | <b>CAG26706.1</b>  |  |          |
| $\alpha$ -2,8-sialyltransferase (GD3 Synthase) ST8Sia                 |          | <i>Gallus gallus</i> | 2.4.99.- | U73176   | AAC28888.1   | <b>P79783</b>  |          |
| $\alpha$ -2,8-sialyltransferase (SIAT8B)                              |          | <i>Gallus gallus</i> | n.d.     | AJ699419   | <b>CAG27881.1</b>  |  |          |
| $\alpha$ -2,8-sialyltransferase (SIAT8C)                              |          | <i>Gallus gallus</i> | n.d.     | AJ699420   | <b>CAG27882.1</b>  |  |          |
| $\alpha$ -2,8-sialyltransferase (SIAT8F)                              |          | <i>Gallus gallus</i> | n.d.     | AJ699424   | <b>CAG27886.1</b>  |  |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia $\alpha$ -V (SIAT8C)           |          | <i>Gallus gallus</i> | n.d.     | AJ704564   | <b>CAG28697.1</b>  |  |          |
| $\beta$ -galactosamide $\alpha$ -2,6-sialyltransferase II (ST6Gal II) |          | <i>Gallus gallus</i> | n.d.     | AJ627629   | <b>CAF29497.1</b>  |  |          |
| GM3 synthase (SIAT9)  |          | <i>Gallus gallus</i> | 2.4.99.9 | AY515255   | <b>AAS83519.1</b>  |  |          |
| polysialyltransferase ST8Sia IV                                       |          | <i>Gallus gallus</i> | 2.4.99.- | AF008194   | AAB95120.1   | <b>O42399</b>  |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal I                              |          | <i>Homo sapiens</i>  | 2.4.99.4 | L29555<br>AF059321<br>L13972<br>AF155238<br>AF186191<br>BC018357<br>NM_003033<br>NM_173344   | AAA36612.1<br>AAC17874.1<br>AAC37574.1<br>AAD39238.1<br><b>AAG29876.1</b><br>AAH18357.1<br>NP_003024.1<br>NP_775479.1  | Q11201<br>O60677<br>Q9UN51   |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal II                             |          | <i>Homo sapiens</i>  | 2.4.99.4 | U63090<br>BC036777<br>X96667<br>NM_006927  | AAB40389.1<br><b>AAH36777.1</b><br>CAA65447.1<br>NP_008858.1   | Q16842<br>O00654   |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal III (SiaT6)                    |          | <i>Homo sapiens</i>  | 2.4.99.6 | L23768<br>BC050380<br>AF425851<br>AF425852<br>AF425853<br>AF425854<br>AF425855<br>AF425856<br>AF425857<br>AF425858<br>AF425859<br>AF425860<br>AF425861<br>AF425862<br>AF425863<br>AF425864<br>AF425865<br>AF425866<br>AF425867<br>AY167992<br>AY167993<br>AY167994 | AAA35778.1<br>AAH50380.1<br>AAO13859.1<br>AAO13860.1<br>AAO13861.1<br>AAO13862.1<br>AAO13863.1<br>AAO13864.1<br>AAO13865.1<br>AAO13866.1<br>AAO13867.1<br>AAO13868.1<br>AAO13869.1<br><b>AAO13870.1</b><br>AAO13871.1<br>AAO13872.1<br>AAO13873.1<br>AAO13874.1<br>AAO13875.1<br>AO38806.1<br>AO38807.1<br>AO38808.1 | Q11203<br>Q86UR6<br>Q86UR7<br>Q86UR8<br>Q86UR9<br>Q86US0<br>Q86US1<br>Q86US2<br>Q8IX43<br>Q8IX44<br>Q8IX45<br>Q8IX46<br>Q8IX47<br>Q8IX48<br>Q8IX49<br>Q8IX50<br>Q8IX51<br>Q8IX52<br>Q8IX53<br>Q8IX54<br>Q8IX55<br>Q8IX56 |          |

FIG. 2C

| Protein  | Organism            | EC#      | GenBank / GenPept  |  | SwissProt  | PDB / 3D |
|--|---------------------|----------|--|--|--|----------|
|  |                     |          | AY167995<br>AY167996<br>AY167997<br>AY167998<br>NM_006279<br>NM_174964<br>NM_174965<br>NM_174966<br>NM_174967<br>NM_174969<br>NM_174970<br>NM_174972 | AAO38809.1<br>AAO38810.1<br>AAO38811.1<br>AAO38812.1<br>NP_006270.1<br>NP_777624.1<br>NP_777625.1<br>NP_777626.1<br>NP_777627.1<br>NP_777629.1<br>NP_777630.1<br>NP_777632.1 | Q8IX57<br>Q8IX58   |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal IV              | <i>Homo sapiens</i> | 2.4.99.- | L23767<br>AF035249<br>BC010645<br>AY040826<br>AF516602<br>AF516603<br>AF516604<br>AF525084<br>X74570<br>CR456858<br>NM_006278                        | AAA16460.1<br>AAC14162.1<br><b>AAH10645.1</b><br>AAK93790.1<br>AAM66431.1<br>AAM66432.1<br>AAM66433.1<br>AAM81378.1<br>CAA52662.1<br>CAG33139.1<br>NP_006269.1               | Q11206<br>O60497<br>Q96QQ9<br>Q8N6A6<br>Q8N6A7<br>Q8NFD3<br>Q8NFG7 |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal VI              | <i>Homo sapiens</i> | 2.4.99.4 | AF119391<br>BC023312<br>AB022918<br>AX877828<br>AX886023<br>NM_006100  | <b>AAD39131.1</b><br>AAH23312.1<br>BAA77609.1<br>CAE89895.1<br>CAF00161.1<br>NP_006091.1   | Q9Y274   |          |
| $\alpha$ -2,6-sialyltransferase (ST6Gal II ; KIAA1877) | <i>Homo sapiens</i> | n.d.     | BC008680<br>AB058780<br>AB059555<br>AJ512141<br>AX795193<br>AX795193<br>NM_032528  | AAH08680.1<br><b>BAB47506.1</b><br>BAC24793.1<br>CAD54408.1<br>CAE48260.1<br>CAE48261.1<br>NP_115917.1   | Q86Y44<br>Q8IUG7<br>Q96HE4<br>Q96JF0                               |          |
| $\alpha$ -2,6-sialyltransferase (ST6GALNAC III)        | <i>Homo sapiens</i> | n.d.     | BC059363<br>AY358540<br>AK091215<br>AJ507291<br>NM_152996  | AAH59363.1<br>AAQ88904.1<br>BAC03611.1<br><b>CAD45371.1</b><br>NP_694541.1   | Q8N259<br>Q8NDV1   |          |
| $\alpha$ -2,6-sialyltransferase (ST6GalNAc V)          | <i>Homo sapiens</i> | n.d.     | BC001201<br>AK056241<br>AL035409<br>AJ507292<br>NM_030965  | <b>AAH01201.1</b><br>BAB71127.1<br>CAB72344.1<br>CAD45372.1<br>NP_112227.1   | Q9BVH7   |          |
| $\alpha$ -2,6-sialyltransferase (SThM) ST6GalNAc II    | <i>Homo sapiens</i> | 2.4.99.- | U14550<br>BC040455<br>AJ251053<br>NM_006456  | AAA52228.1<br><b>AAH40455.1</b><br>CAB61434.1<br>NP_006447.1   | Q9UJ37<br>Q12971   |          |
| $\alpha$ -2,6-sialyltransferase ST6Gal I               | <i>Homo sapiens</i> | 2.4.99.1 | BC031476<br>BC040009<br>A17362<br>A23699<br>X17247<br>X54363<br>X62822<br>NM_003032<br>NM_173216   | AAH31476.1<br>AAH40009.1<br><b>CAA01327.1</b><br>CAA01686.1<br>CAA35111.1<br>CAA38246.1<br>CAA44634.1<br>NP_003023.1<br>NP_775323.1  | P15907   |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc I            | <i>Homo sapiens</i> | 2.4.99.3 | BC022462<br>AY096001<br>AY358918<br>AK000113<br>Y11339   | AAH22462.1<br>AAM22800.1<br>AAQ89277.1<br>BAA90953.1<br><b>CAA72179.2</b>  | Q8TBJ6<br>Q9NSC7<br>Q9NXQ7   |          |

FIG. 2D

| Protein   | Organism            | EC#      | GenBank / GenPept   |   | SwissProt  | PDB / 3D |
|---|---------------------|----------|---|---|--|----------|
| $\alpha$ -2,8-polysialyltransferase ST8Sia IV                                     | <i>Homo sapiens</i> | 2.4.99.- | NM_018414<br>L41680<br>BC027866<br>BC053657<br>NM_005668  | NP_060884.1<br>AAC41775.1<br>AAH27866.1<br><b>AAH53657.1</b><br>NP_005659.1   |  |          |
| $\alpha$ -2,8-sialyltransferase (GD3 synthase) ST8Sia I                           | <i>Homo sapiens</i> | 2.4.99.8 | L32867<br>L43494<br>BC046158<br>-<br>AY569975<br>D26360<br>X77922<br>NM_003034  | AAA62366.1<br><b>AAC37586.1</b><br>AAH46158.1<br>AAQ53140.1<br>AAS75783.1<br>BAA05391.1<br>CAA54891.1<br>NP_003025.1  | Q86X71<br>Q92185<br>Q93064                               |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia II   | <i>Homo sapiens</i> | 2.4.99.- | L29556<br>U82762<br>U33551<br>BC069584<br>NM_006011   | AAA36613.1<br>AAB51242.1<br><b>AAC24458.1</b><br>AAH69584.1<br>NP_006002.1  | Q92186<br>Q92470<br>Q92746                               |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia III  | <i>Homo sapiens</i> | 2.4.99.- | AF004668<br>AF003092<br>NM_015879   | <b>AAB87642.1</b><br>AAC15901.2<br>NP_056963.1  | O43173<br>Q9NS41   |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia V  | <i>Homo sapiens</i> | 2.4.99.- | U91641<br>CR457037<br>NM_013305   | <b>AAC51727.1</b><br>CAG33318.1<br>NP_037437.1  | O15466   |          |
| ENSP0000020221 (fragment)   |                     | n.d.     | AC023295  | -   |  |          |
| lactosylceramide $\alpha$ -2,3-sialyltransferase (ST3Gal V)                       | <i>Homo sapiens</i> | 2.4.99.9 | AF105026<br>AF119415<br>BC065936<br>AY152815<br>AAP65066<br>AY359105<br>AB018356<br>AX876536<br>NM_003896                             | <b>AAD14634.1</b><br>AAF66146.1<br>AAH65936.1<br>AAO16866.1<br>AAP65066.1<br>AAQ89463.1<br>BAA33950.1<br>CAE89320.1<br>NP_003887.2  | Q9UNP4<br>O94902   |          |
| <i>N</i> -acetylgalactosaminide $\alpha$ -2,6-sialyltransferase (ST6GalNAc VI)    | <i>Homo sapiens</i> | 2.4.99.- | BC006564<br>BC007802<br>BC016299<br>AY358672<br>AB035173<br>AK023900<br>AJ507293<br>AX880950<br>CR457318<br>NM_013443                 | AAH06564.1<br>AAH07802.1<br>AAH16299.1<br>AAQ89035.1<br><b>BAA87035.1</b><br>BAB14715.1<br>CAD45373.1<br>CAE91145.1<br>CAG33599.1<br>NP_038471.2                              | Q969X2<br>Q9H8A2<br>Q9ULB8                               |          |
| <i>N</i> -acetylgalactosaminide $\alpha$ -2,6-sialyltransferase IV (ST6GalNAc IV) | <i>Homo sapiens</i> | 2.4.99.- | AF127142<br>BC036705<br>-<br>AB035172<br>AK000600<br>Y17461<br>AJ271734<br>AX061620<br>AX068265<br>AX969252<br>NM_014403<br>NM_175039 | AAF00102.1<br>AAH36705.1<br>AAP63349.1<br><b>BAA87034.1</b><br>BAA91281.1<br>CAB44354.1<br>CAC07404.1<br>CAC24981.1<br>CAC27250.1<br>CAF14360.1<br>NP_055218.3<br>NP_778204.1 | Q9H4F1<br>Q9NWU6<br>Q9UKU1<br>Q9ULB9<br>Q9Y3G3<br>Q9Y3G4 |          |
| ST8SIA-VI (fragment)  | <i>Homo sapiens</i> | n.d.     | AJ621583<br>XM_291725   | <b>CAF21722.1</b><br>XP_291725.2  |  |          |
| unnamed protein product   | <i>Homo sapiens</i> | n.d.     | AK021929<br>AX881696  | <b>BAB13940.1</b><br>CAE91353.1   | Q9HAA9   |          |
| Gal $\beta$ -1,3/4-GlcNAc $\alpha$ -  | <i>Mesocricetus</i> | 2.4.99.6 | AJ245699  | <b>CAB53394.1</b>   | Q9QXF6   |          |

FIG. 2E

| Protein   |                   | Organism                    | EC#      | GenBank / GenPept  |  | SwissProt                                      | PDB / 3D |
|---|-------------------|-----------------------------|----------|--|--|--|----------|
| 2,3-sialyltransferase (ST3Gal III)                                    |                   | <i>auratus</i>              |          |  |  |  |          |
| Gal $\beta$ -1,3/4-GlcNAc $\alpha$ -2,3-sialyltransferase (ST3Gal IV) |                   | <i>Mesocricetus auratus</i> | 2.4.99.6 | AJ245700   | <b>CAB53395.1</b>  | Q9QXF5   |          |
| GD3 synthase (fragment) ST8Sia I                                      |                   | <i>Mesocricetus auratus</i> | n.d.     | AF141657   | <b>AAD33879.1</b>  | Q9WUL1   |          |
| polysialyltransferase (ST8Sia IV)                                     |                   | <i>Mesocricetus auratus</i> | 2.4.99.- | AJ245701   | <b>CAB53396.1</b>  | Q9QXF4   |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal I                              | <i>St3gal1</i>    | <i>Mus musculus</i>         | 2.4.99.4 | AF214028<br>AK031344<br>AK078469<br>X73523<br>NM_009177                                      | AAF60973.1<br>BAC27356.1<br>BAC37290.1<br>CAA51919.1<br>NP_033203.1  | <b>P54751</b><br><b>Q11202</b><br>Q9JL30       |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal II                             | <i>St3gal2</i>    | <i>Mus musculus</i>         | 2.4.99.4 | BC015264<br>BC066064<br>AK034554<br>AK034863<br>AK053827<br>X76989<br>NM_009179<br>NM_178048 | AAH15264.1<br>AAH66064.1<br>BAC28752.1<br>BAC28859.1<br>BAC35543.1<br>CAA54294.1<br>NP_033205.1<br>NP_835149.1 | Q11204<br>Q8BPL0<br>Q8BSA0<br>Q8BSE9<br>Q91WH6 |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal III                            | <i>St3gal3</i>    | <i>Mus musculus</i>         | 2.4.99.- | BC006710<br>AK005053<br>AK013016<br>X84234<br>NM_009176                                      | AAH06710.1<br><b>BAB23779.1</b><br>BAB28598.1<br>CAA59013.1<br>NP_033202.2                                     | P97325<br>Q922X5<br>Q9CZ48<br>Q9DBB6           |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal IV                             | <i>St3gal4</i>    | <i>Mus musculus</i>         | 2.4.99.4 | BC011121<br>BC050773<br>D28941<br>AK008543<br>AB061305<br>X95809<br>NM_009178                | AAH11121.1<br><b>AAH50773.1</b><br>BAA06068.1<br>BAB25732.1<br>BAB47508.1<br>CAA65076.1<br>NP_033204.2         | P97354<br>Q61325<br>Q91Y74<br>Q921R5<br>Q9CVE8 |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal VI                             | <i>St3gal6</i>    | <i>Mus musculus</i>         | 2.4.99.4 | AF119390<br>BC052338<br>AB063326<br>AK033562<br>AK041173<br>NM_018784                        | AAD39130.1<br>AAH52338.1<br><b>BAB79494.1</b><br>BAC28360.1<br>BAC30851.1<br>NP_061254                         | Q80UR7<br>Q8BLV1<br>Q8VIB3<br>Q9WVG2           |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc II                          | <i>St6galnac2</i> | <i>Mus musculus</i>         | 2.4.99.- | NM_009180<br>BC010208<br>AB027198<br>AK004613<br>X93999<br>X94000<br>NM_009180               | 6677963<br>AAH10208.1<br>BAB00637.1<br>BAB23410.1<br>CAA63821.1<br>CAA63822.1<br>NP_033206.2                   | <b>P70277</b><br>Q9DC24<br>Q9JJM5              |          |
| $\alpha$ -2,6-sialyltransferase ST6Gal I                              | <i>St6gal1</i>    | <i>Mus musculus</i>         | 2.4.99.1 | -<br>BC027833<br>D16106<br>AK034768<br>AK084124<br>NM_145933                                 | AAE68031.1<br>AAH27833.1<br>BAA03680.1<br>BAC28828.1<br>BAC39120.1<br>NP_666045.1                              | <b>Q64685</b><br>Q8BM62<br>Q8K1L1              |          |
| $\alpha$ -2,6-sialyltransferase ST6Gal II                             | <i>St6gal2</i>    | <i>Mus musculus</i>         | n.d.     | AK082566<br>AB095093<br>AK129462<br>NM_172829  | BAC38534.1<br><b>BAC87752.1</b><br>BAC98272.1<br>NP_766417.1   | Q8BUU4   |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc I                           | <i>St6galnac1</i> | <i>Mus musculus</i>         | 2.4.99.3 | Y11274<br>NM_011371  | <b>CAA72137.1</b><br>NP_035501.1   | Q9QZ39<br>Q9JJP5                               |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc III                         | <i>St6galnac3</i> | <i>Mus musculus</i>         | n.d.     | BC058387<br>AK034804<br>Y11342<br>Y11343   | AAH58387.1<br>BAC28836.1<br>CAA72181.2<br><b>CAB95031.1</b>  | Q9WUV2<br>Q9JHP5                               |          |

FIG. 2F

| Protein  | Organism          |                     | EC#      | GenBank / GenPept  |   | SwissProt  | PDB / 3D |
|--|-------------------|---------------------|----------|--|---|--|----------|
| $\alpha$ -2,6-sialyltransferase ST6GalNAc IV                                   | <i>St6galnac4</i> | <i>Mus musculus</i> | 2.4.99.7 | NM_011372<br>BC056451<br>AK085730<br>AJ007310<br>Y15779<br>Y15780<br>Y19055<br>Y19057<br>NM_011373 | NP_035502<br>AAH56451.1<br>BAC39523.1<br>CAA07446.1<br>CAB43507.1<br><b>CAB43514.1</b><br>CAB93946.1<br>CAB93948.1<br>NP_035503.1 | Q8C3J2<br>Q9JHP2<br>Q9R2B6<br>Q88725<br>Q9JHP0<br>Q9QUP9<br>Q9R2B5 |          |
| $\alpha$ -2,8-sialyltransferase (GD3 synthase) ST8Sia I                        | <i>St8sia1</i>    | <i>Mus musculus</i> | 2.4.99.8 | L38677<br>BC024821<br>AK046188<br>AK052444<br>X84235<br>AJ401102<br>NM_011374                      | AAA91869.1<br><b>AAH24821.1</b><br>BAC32625.1<br>BAC34994.1<br>CAA59014.1<br>CAC20706.1<br>NP_035504.1                            | Q64468<br>Q64687<br>Q8BL76<br>Q8BWI0<br>Q8K1C1<br>Q9EPK0           |          |
| $\alpha$ -2,8-sialyltransferase (ST8Sia VI)                                    | <i>St8sia6</i>    | <i>Mus musculus</i> | n.d.     | AB059554<br>AK085105<br>NM_145838  | <b>BAC01265.1</b><br>BAC39367.1<br>NP_665837.1  | Q8BI43<br>Q8K4T1   |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia II                                      | <i>St8sia2</i>    | <i>Mus musculus</i> | 2.4.99.- | X83562<br>X99646<br>X99647<br>X99648<br>X99649<br>X99650<br>X99651<br>NM_009181                    | CAA58548.1<br>CAA67965.1<br>CAA67965.1<br>CAA67965.1<br>CAA67965.1<br>CAA67965.1<br>CAA67965.1<br>NP_033207.1                     | O35696   |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia IV                                      | <i>St8sia4</i>    | <i>Mus musculus</i> | 2.4.99.8 | BC060112<br>AK003690<br>AK041723<br>AJ223956<br>X86000<br>Y09484<br>NM_009183                      | AAH60112.1<br>BAB22941.1<br>BAC31044.1<br><b>CAA11685.1</b><br>CAA59992.1<br>CAA70692.1<br>NP_033209.1                            | Q64692<br>Q8BY70   |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia V                                       | <i>St8sia5</i>    | <i>Mus musculus</i> | 2.4.99.- | BC034855<br>AK078670<br>X98014<br>X98014<br>X98014<br>NM_013666<br>NM_153124<br>NM_177416          | AAH34855.1<br>BAC37354.1<br><b>CAA66642.1</b><br>CAA66643.1<br>CAA66644.1<br>NP_038694.1<br>NP_694764.1<br>NP_803135.1            | P70126<br>P70127<br>P70128<br>Q8BJW0<br>Q8JZQ3                     |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia III                                     | <i>St8sia3</i>    | <i>Mus musculus</i> | 2.4.99.- | BC075645<br>AK015874<br>X80502<br>NM_009182  | AAH75645.1<br>BAB30012.1<br>CAA56665.1<br>NP_033208.1   | Q64689<br>Q9CUJ6   |          |
| GD1 synthase (ST6GalNAc V)   | <i>St6galnac5</i> | <i>Mus musculus</i> | n.d.     | BC055737<br>AB030836<br>AB028840<br>AK034387<br>AK038434<br>AK042683<br>NM_012028                  | <b>AAH55737.1</b><br>BAA85747.1<br>BAA89292.1<br>BAC28693.1<br>BAC29997.1<br>BAC31331.1<br>NP_036158.2                            | Q8CAM7<br>Q8CBX1<br>Q9QYJ1<br>Q9R0K6                               |          |
| GM3 synthase ( $\alpha$ -2,3-sialyltransferase) ST3Gal V                       | <i>St3gal5</i>    | <i>Mus musculus</i> | 2.4.99.9 | AF119416<br>AB018048<br>AB013302<br>AK012961<br>Y15003<br>NM_011375                                | <b>AAF66147.1</b><br>AAP65063.1<br>BAA33491.1<br>BAA76467.1<br>BAB28571.1<br>CAA75235.1<br>NP_035505.1                            | O88829<br>Q9CZ65<br>Q9QWF9   |          |
| <i>N</i> -acetylgalactosaminide $\alpha$ -2,6-sialyltransferase (ST6GalNAc VI) | <i>St6galnac6</i> | <i>Mus musculus</i> | 2.4.99.- | BC036985<br>AB035174<br>AB035123<br>AK030648   | <b>AAH36985.1</b><br>BAA87036.1<br>BAA95940.1<br>BAC27064.1   | Q8CDC3<br>Q8JZW3<br>Q9JM95<br>Q9R0G9                               |          |

FIG. 2G

| Protein  | Organism                                      | EC#      | GenBank / GenPept                            |   | SwissProt | PDB / 3D |
|--|---|----------|--|---|-----------|----------|
| M138L  | <i>Myxoma virus</i>                           | n.d.     | NM_016973<br>U46578<br>AF170726<br>NC_001132 | NP_058669.1<br>AAD00069.1<br>AAE61323.1<br>AAE61326.1<br><b>AAF15026.1</b><br>NP_051852.1 |           |          |
| $\alpha$ -2,3-sialyltransferase (St3Gal-I)                       | <i>Oncorhynchus mykiss</i>                    | n.d.     | AJ585760                                     | <b>CAE51384.1</b>   |           |          |
| $\alpha$ -2,6-sialyltransferase (Siat1)                          | <i>Oncorhynchus mykiss</i>                    | n.d.     | AJ620649                                     | <b>CAF05848.1</b>   |           |          |
| $\alpha$ -2,8-polysialyltransferase IV (ST8Sia IV)               | <i>Oncorhynchus mykiss</i>                    | n.d.     | AB094402                                     | <b>BAC77411.1</b>   | Q7T2X5    |          |
| GalNAc $\alpha$ -2,6-sialyltransferase (RtST6GalNAc)             | <i>Oncorhynchus mykiss</i>                    | n.d.     | AB097943                                     | <b>BAC77520.1</b>   | Q7T2X4    |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal IV                        | <i>Oryctolagus cuniculus</i>                  | 2.4.99.- | AF121967                                     | <b>AAF28871.1</b>   | Q9N257    |          |
| OJ1217_F02.7   | <i>Oryza sativa (japonica cultivar-group)</i> | n.d.     | AP004084                                     | <b>BAD07616.1</b>   |           |          |
| OSJNBa0043L24.2 or OSJNBb0002J11.9                               | <i>Oryza sativa (japonica cultivar-group)</i> | n.d.     | AL731626<br>AL662969                         | <b>CAD41185.1</b><br>CAE04714.1   |           |          |
| P0683f02.18 or P0489B03.1  | <i>Oryza sativa (japonica cultivar-group)</i> | n.d.     | AP003289<br>AP003794                         | <b>BAB63715.1</b><br>BAB90552.1   |           |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc V (Siat7E) (fragment)  | <i>Oryzias latipes</i>                        | n.d.     | AJ646876                                     | <b>CAG26705.1</b>   |           |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal I (Siat4)                 | <i>Pan troglodytes</i>                        | n.d.     | AJ744803                                     | <b>CAG32839.1</b>   |           |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal II (Siat5)                | <i>Pan troglodytes</i>                        | n.d.     | AJ744804                                     | <b>CAG32840.1</b>   |           |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal III (Siat6)               | <i>Pan troglodytes</i>                        | n.d.     | AJ626819                                     | <b>CAF25177.1</b>   |           |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal IV (Siat4c)               | <i>Pan troglodytes</i>                        | n.d.     | AJ626824                                     | <b>CAF25182.1</b>   |           |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal VI (Siat10)               | <i>Pan troglodytes</i>                        | n.d.     | AJ744808                                     | <b>CAG32844.1</b>   |           |          |
| $\alpha$ -2,6-sialyltransferase (Sia7A)                          | <i>Pan troglodytes</i>                        | n.d.     | AJ748740                                     | <b>CAG38615.1</b>   |           |          |
| $\alpha$ -2,6-sialyltransferase (Sia7B)                          | <i>Pan troglodytes</i>                        | n.d.     | AJ748741                                     | <b>CAG38616.1</b>   |           |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc III (Siat7C)           | <i>Pan troglodytes</i>                        | n.d.     | AJ634454                                     | <b>CAG25676.1</b>   |           |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc IV (Siat7D) (fragment) | <i>Pan troglodytes</i>                        | n.d.     | AJ646870                                     | <b>CAG26699.1</b>   |           |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc V (Siat7E)             | <i>Pan troglodytes</i>                        | n.d.     | AJ646875                                     | <b>CAG26704.1</b>   |           |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc VI (Siat7F) (fragment) | <i>Pan troglodytes</i>                        | n.d.     | AJ646882                                     | <b>CAG26711.1</b>   |           |          |
| $\alpha$ -2,8-sialyltransferase 8A (Siat8A)                      | <i>Pan troglodytes</i>                        | 2.4.99.8 | AJ697658                                     | <b>CAG26896.1</b>   |           |          |
| $\alpha$ -2,8-sialyltransferase 8B (Siat8B)                      | <i>Pan troglodytes</i>                        | n.d.     | AJ697659                                     | <b>CAG26897.1</b>   |           |          |
| $\alpha$ -2,8-sialyltransferase 8C (Siat8C)                      | <i>Pan troglodytes</i>                        | n.d.     | AJ697660                                     | <b>CAG26898.1</b>   |           |          |
| $\alpha$ -2,8-sialyltransferase 8D (Siat8D)                      | <i>Pan troglodytes</i>                        | n.d.     | AJ697661                                     | <b>CAG26899.1</b>   |           |          |
| $\alpha$ -2,8-sialyltransferase                                  | <i>Pan troglodytes</i>                        | n.d.     | AJ697662                                     | <b>CAG26900.1</b>   |           |          |

FIG. 2H

| Protein  | Organism                          | EC#      | GenBank / GenPept               |   | SwissProt                | PDB / 3D |
|--|-----------------------------------|----------|---------------------------------|---|--------------------------|----------|
| 8E (Siat8E)  |                                   |          |                                 |   |                          |          |
| $\alpha$ -2,8-sialyltransferase 8F (Siat8F)                                | <i>Pan troglodytes</i>            | n.d.     | AJ697663                        | <b>CAG26901.1</b>                       |                          |          |
| $\beta$ -galactosamide $\alpha$ -2,6-sialyltransferase I (ST6Gal I; Siat1) | <i>Pan troglodytes</i>            | 2.4.99.1 | AJ627624                        | <b>CAF29492.1</b>                       |                          |          |
| $\beta$ -galactosamide $\alpha$ -2,6-sialyltransferase II (ST6Gal II)      | <i>Pan troglodytes</i>            | n.d.     | AJ627625                        | <b>CAF29493.1</b>                       |                          |          |
| GM3 synthase ST3Gal V (Siat9)  | <i>Pan troglodytes</i>            | n.d.     | AJ744807                        | <b>CAG32843.1</b>                       |                          |          |
| S138L  | <i>Rabbit fibroma virus Kasza</i> | n.d.     | NC_001266                       | <b>NP_052025</b>                        |                          |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal III                                 | <i>Rattus norvegicus</i>          | 2.4.99.6 | M97754<br>NM_031697             | AAA42146.1<br>NP_113885.1               | <b>Q02734</b>            |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal IV (Siat4c)                         | <i>Rattus norvegicus</i>          | n.d.     | AJ626825                        | <b>CAF25183.1</b>                       |                          |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal VI                                  | <i>Rattus norvegicus</i>          | n.d.     | AJ626743                        | <b>CAF25053.1</b>                       |                          |          |
| $\alpha$ -2,6-sialyltransferase ST3Gal II                                  | <i>Rattus norvegicus</i>          | 2.4.99.- | X76988<br>NM_031695             | CAA54293.1<br>NP_113883.1               | <b>Q11205</b>            |          |
| $\alpha$ -2,6-sialyltransferase ST6Gal I                                   | <i>Rattus norvegicus</i>          | 2.4.99.1 | M18769<br>M83143                | AAA41196.1<br>AAB07233.1                | <b>P13721</b>            |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc I (Siat7A)                       | <i>Rattus norvegicus</i>          | n.d.     | AJ634458                        | <b>CAG25684.1</b>                       |                          |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc II (Siat7B)                      | <i>Rattus norvegicus</i>          | n.d.     | AJ634457                        | <b>CAG25679.1</b>                       |                          |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc III                              | <i>Rattus norvegicus</i>          | 2.4.99.- | L29554<br>BC072501<br>NM_019123 | AAC42086.1<br>AAH72501.1<br>NP_061996.1 | <b>Q64686</b>            |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc IV (Siat7D) (fragment)           | <i>Rattus norvegicus</i>          | n.d.     | AJ646871                        | <b>CAG26700.1</b>                       |                          |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc V (Siat7E)                       | <i>Rattus norvegicus</i>          | n.d.     | AJ646872                        | <b>CAG26701.1</b>                       |                          |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc VI (Siat7F) (fragment)           | <i>Rattus norvegicus</i>          | n.d.     | AJ646881                        | <b>CAG26710.1</b>                       |                          |          |
| $\alpha$ -2,8-sialyltransferase (GD3 synthase) ST8Sia                      | <i>Rattus norvegicus</i>          | 2.4.99.- | U53883<br>D45255                | AAC27541.1<br>BAA08213.1                | <b>P70554<br/>P97713</b> |          |
| $\alpha$ -2,8-sialyltransferase (SIAT8E)                                   | <i>Rattus norvegicus</i>          | n.d.     | AJ699422                        | <b>CAG27884.1</b>                       |                          |          |
| $\alpha$ -2,8-sialyltransferase (SIAT8F)                                   | <i>Rattus norvegicus</i>          | n.d.     | AJ699423                        | <b>CAG27885.1</b>                       |                          |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia II                                  | <i>Rattus norvegicus</i>          | 2.4.99.- | L13445<br>NM_057156             | AAA42147.1<br>NP_476497.1               | <b>Q07977<br/>Q64688</b> |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia III                                 | <i>Rattus norvegicus</i>          | 2.4.99.- | U55938<br>NM_013029             | AAB50061.1<br>NP_037161.1               | <b>P97877</b>            |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia IV                                  | <i>Rattus norvegicus</i>          | 2.4.99.- | U90215                          | AAB49989.1                              | <b>O08563</b>            |          |
| $\beta$ -galactosamide $\alpha$ -2,6-sialyltransferase II (ST6Gal II)      | <i>Rattus norvegicus</i>          | n.d.     | AJ627626                        | <b>CAF29494.1</b>                       |                          |          |
| GM3 synthase ST3Gal V  | <i>Rattus norvegicus</i>          | n.d.     | AB018049<br>NM_031337           | BAA33492.1<br>NP_112627.1               | <b>O88830</b>            |          |

FIG. 2I

| Protein  | Organism                             | EC#      | GenBank / GenPept  |                                 | SwissProt     | PDB / 3D |
|--|--------------------------------------|----------|--------------------|---------------------------------|---------------|----------|
| sialyltransferase ST3Gal-I (Siat4A)                                  | <i>Rattus norvegicus</i>             | n.d.     | AJ748840           | <b>CAG44449.1</b>               |               |          |
| $\alpha$ -2,3-sialyltransferase (St3Gal-II)                          | <i>Silurana tropicalis</i>           | n.d.     | AJ585763           | <b>CAE51387.1</b>               |               |          |
| $\alpha$ -2,6-sialyltransferase (Siat7b)                             | <i>Silurana tropicalis</i>           | n.d.     | AJ620650           | <b>CAF05849.1</b>               |               |          |
| $\alpha$ -2,6-sialyltransferase (St6galnac)                          | <i>Strongylocentrotus purpuratus</i> | n.d.     | AJ699425           | <b>CAG27887.1</b>               |               |          |
| $\alpha$ -2,3-sialyltransferase (ST3GAL-III)                         | <i>Sus scrofa</i>                    | n.d.     | AJ585765           | <b>CAE51389.1</b>               |               |          |
| $\alpha$ -2,3-sialyltransferase (ST3GAL-IV)                          | <i>Sus scrofa</i>                    | n.d.     | AJ584674           | <b>CAE48299.1</b>               |               |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal I                             | <i>Sus scrofa</i>                    | 2.4.99.4 | M97753             | AAA31125.1                      | <b>Q02745</b> |          |
| $\alpha$ -2,6-sialyltransferase (fragment) ST6Gal I                  | <i>Sus scrofa</i>                    | 2.4.99.1 | AF136746           | <b>AAD33059.1</b>               | Q9XSG8        |          |
| $\beta$ -galactosamide $\alpha$ -2,6-sialyltransferase (ST6GalNAc-V) | <i>Sus scrofa</i>                    | n.d.     | AJ620948           | <b>CAF06585.2</b>               |               |          |
| sialyltransferase (fragment) ST6Gal I                                | <i>Sus scrofa</i>                    | n.d.     | AF041031           | <b>AAC15633.1</b>               | O62717        |          |
| ST6GALNAC-V  | <i>Sus scrofa</i>                    | n.d.     | AJ620948           | <b>CAF06585.1</b>               |               |          |
| $\alpha$ -2,3-sialyltransferase (Siat5-r)                            | <i>Takifugu rubripes</i>             | n.d.     | AJ744805           | <b>CAG32841.1</b>               |               |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal I (Siat4)                     | <i>Takifugu rubripes</i>             | n.d.     | AJ626816           | <b>CAF25174.1</b>               |               |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal II (Siat5) (fragment)         | <i>Takifugu rubripes</i>             | n.d.     | AJ626817           | <b>CAF25175.1</b>               |               |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal III (Siat6)                   | <i>Takifugu rubripes</i>             | n.d.     | AJ626818           | <b>CAF25176.1</b>               |               |          |
| $\alpha$ -2,6-sialyltransferase ST6Gal I (Siat1)                     | <i>Takifugu rubripes</i>             | n.d.     | AJ744800           | <b>CAG32836.1</b>               |               |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc II (Siat7B)                | <i>Takifugu rubripes</i>             | n.d.     | AJ634460           | <b>CAG25681.1</b>               |               |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc II B (Siat7B-related)      | <i>Takifugu rubripes</i>             | n.d.     | AJ634461           | <b>CAG25682.1</b>               |               |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc III (Siat7C) (fragment)    | <i>Takifugu rubripes</i>             | n.d.     | AJ634456           | <b>CAG25678.1</b>               |               |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc IV (siat7D) (fragment)     | <i>Takifugu rubripes</i>             | 2.4.99.3 | Y17466<br>AJ646869 | CAB44338.1<br><b>CAG26698.1</b> | Q9W6U6        |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc V (Siat7E) (fragment)      | <i>Takifugu rubripes</i>             | n.d.     | AJ646873           | <b>CAG26702.1</b>               |               |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc VI (Siat7F) (fragment)     | <i>Takifugu rubripes</i>             | n.d.     | AJ646880           | <b>CAG26709.1</b>               |               |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia I (Siat 8A) (fragment)        | <i>Takifugu rubripes</i>             | n.d.     | AJ715534           | <b>CAG29373.1</b>               |               |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia II (Siat 8B) (fragment)       | <i>Takifugu rubripes</i>             | n.d.     | AJ715538           | <b>CAG29377.1</b>               |               |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia III (Siat 8C) (fragment)      | <i>Takifugu rubripes</i>             | n.d.     | AJ715541           | <b>CAG29380.1</b>               |               |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia IIIr (Siat 8Cr)               | <i>Takifugu rubripes</i>             | n.d.     | AJ715542           | <b>CAG29381.1</b>               |               |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia V (Siat 8E)                   | <i>Takifugu rubripes</i>             | n.d.     | AJ715547           | <b>CAG29386.1</b>               |               |          |

FIG. 2J

| Protein   | Organism                      | EC#      | GenBank / GenPept                |  | SwissProt | PDB / 3D |
|---|-------------------------------|----------|----------------------------------|--|-----------|----------|
| (fragment)  |                               |          |                                  |  |           |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia VI (Siat 8F) (fragment)        | <i>Takifugu rubripes</i>      | n.d.     | AJ715549                         | CAG29388.1                             |           |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia Vlr (Siat 8Fr)                 | <i>Takifugu rubripes</i>      | n.d.     | AJ715550                         | CAG29389.1                             |           |          |
| $\alpha$ -2,3-sialyltransferase (Siat5-r)                             | <i>Tetraodon nigroviridis</i> | n.d.     | AJ744806                         | CAG32842.1                             |           |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal I (Siat4)                      | <i>Tetraodon nigroviridis</i> | n.d.     | AJ744802                         | CAG32838.1                             |           |          |
| $\alpha$ -2,3-sialyltransferase ST3Gal III (Siat6)                    | <i>Tetraodon nigroviridis</i> | n.d.     | AJ626822                         | CAF25180.1                             |           |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc II (Siat7B)                 | <i>Tetraodon nigroviridis</i> | n.d.     | AJ634462                         | CAG25683.1                             |           |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc V (Siat7E) (fragment)       | <i>Tetraodon nigroviridis</i> | n.d.     | AJ646879                         | CAG26708.1                             |           |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia I (Siat 8A) (fragment)         | <i>Tetraodon nigroviridis</i> | n.d.     | AJ715536                         | CAG29375.1                             |           |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia II (Siat 8B) (fragment)        | <i>Tetraodon nigroviridis</i> | n.d.     | AJ715537                         | CAG29376.1                             |           |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia III (Siat 8C) (fragment)       | <i>Tetraodon nigroviridis</i> | n.d.     | AJ715539                         | CAG29378.1                             |           |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia IIIr (Siat 8Cr) (fragment)     | <i>Tetraodon nigroviridis</i> | n.d.     | AJ715540                         | CAG29379.1                             |           |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia V (Siat 8E) (fragment)         | <i>Tetraodon nigroviridis</i> | n.d.     | AJ715548                         | CAG29387.1                             |           |          |
| $\alpha$ -2,3-sialyltransferase (St3Gal-II)                           | <i>Xenopus laevis</i>         | n.d.     | AJ585762                         | CAE51386.1                             |           |          |
| $\alpha$ -2,3-sialyltransferase (St3Gal-VI)                           | <i>Xenopus laevis</i>         | n.d.     | AJ585766                         | CAE51390.1                             |           |          |
| $\alpha$ -2,3-sialyltransferase St3Gal-III (Siat6)                    | <i>Xenopus laevis</i>         | n.d.     | AJ585764<br>AJ626823             | CAE51388.1<br>CAF25181.1               |           |          |
| $\alpha$ -2,8-polysialyltransferase                                   | <i>Xenopus laevis</i>         | 2.4.99.- | AB007468                         | BAA32617.1                             | O93234    |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia-I (Siat8A;GD3 synthase)        | <i>Xenopus laevis</i>         | n.d.     | AY272056<br>AY272057<br>AJ704562 | AAQ16162.1<br>AAQ16163.1<br>CAG28695.1 |           |          |
| Unknown (protein for MGC:81265)                                       | <i>Xenopus laevis</i>         | n.d.     | BC068760                         | AAH68760.1                             |           |          |
| $\alpha$ -2,3-sialyltransferase (3Gal-VI)                             | <i>Xenopus tropicalis</i>     | n.d.     | AJ626744                         | CAF25054.1                             |           |          |
| $\alpha$ -2,3-sialyltransferase (Siat4c)                              | <i>Xenopus tropicalis</i>     | n.d.     | AJ622908                         | CAF22058.1                             |           |          |
| $\alpha$ -2,6-sialyltransferase ST6GalNAc V (Siat7E) (fragment)       | <i>Xenopus tropicalis</i>     | n.d.     | AJ646878                         | CAG26707.1                             |           |          |
| $\alpha$ -2,8-sialyltransferase ST8Sia III (Siat 8C) (fragment)       | <i>Xenopus tropicalis</i>     | n.d.     | AJ715544                         | CAG29383.1                             |           |          |
| $\beta$ -galactosamide $\alpha$ -2,6-sialyltransferase II (ST6Gal II) | <i>Xenopus tropicalis</i>     | n.d.     | AJ627628                         | CAF29496.1                             |           |          |
| sialyltransferase St8Sial   | <i>Xenopus tropicalis</i>     | n.d.     | AY652775                         | AAT67042                               |           |          |
| poly- $\alpha$ -2,8-sialosyl sialyltransferase (NeuS)                 | <i>Escherichia coli K1</i>    | 2.4.--   | M76370<br>X60598                 | AAA24213.1<br>CAA43053.1               | Q57269    |          |
| polysialyltransferase   | <i>Escherichia coli K92</i>   | 2.4.--   | M88479                           | AAA24215.1                             | Q47404    |          |

FIG. 2K

| Protein                                   | Organism                                      | EC#      | GenBank / GenPept               |   | SwissProt        | PDB / 3D |
|---|---|----------|---------------------------------|---|------------------|----------|
| $\alpha$ -2,8 polysialyltransferase SiaD  | <i>Neisseria meningitidis</i> B1940           | 2.4.-.-  | M95053<br>X78068                | AAA20478.1<br>CAA54985.1                              | Q51281<br>Q51145 |          |
| SynE                                      | <i>Neisseria meningitidis</i> FAM18           | n.d.     | U75650                          | <b>AAB53842.1</b>                                     | O06435           |          |
| polysialyltransferase (SiaD)(fragment)    | <i>Neisseria meningitidis</i> M1019           | n.d.     | AY234192                        | <b>AAO85290.1</b>                                     |                  |          |
| SiaD (fragment)                           | <i>Neisseria meningitidis</i> M209            | n.d.     | AY281046                        | <b>AAP34769.1</b>                                     |                  |          |
| SiaD (fragment)                           | <i>Neisseria meningitidis</i> M3045           | n.d.     | AY281044                        | <b>AAP34767.1</b>                                     |                  |          |
| polysialyltransferase (SiaD)(fragment)    | <i>Neisseria meningitidis</i> M3315           | n.d.     | AY234191                        | <b>AAO85289.1</b>                                     |                  |          |
| SiaD (fragment)                           | <i>Neisseria meningitidis</i> M3515           | n.d.     | AY281047                        | <b>AAP34770.1</b>                                     |                  |          |
| polysialyltransferase (SiaD)(fragment)    | <i>Neisseria meningitidis</i> M4211           | n.d.     | AY234190                        | <b>AAO85288.1</b>                                     |                  |          |
| SiaD (fragment)                           | <i>Neisseria meningitidis</i> M4642           | n.d.     | AY281048                        | <b>AAP34771.1</b>                                     |                  |          |
| polysialyltransferase (SiaD)(fragment)    | <i>Neisseria meningitidis</i> M5177           | n.d.     | AY234193                        | <b>AAO85291.1</b>                                     |                  |          |
| SiaD                                      | <i>Neisseria meningitidis</i> M5178           | n.d.     | AY281043                        | <b>AAP34766.1</b>                                     |                  |          |
| SiaD (fragment)                           | <i>Neisseria meningitidis</i> M980            | n.d.     | AY281045                        | <b>AAP34768.1</b>                                     |                  |          |
| NMB0067                                   | <i>Neisseria meningitidis</i> MC58            | n.d.     | NC_003112                       | <b>NP_273131</b>                                      |                  |          |
| Lst                                       | <i>Aeromonas punctata</i> Sch3                | n.d.     | AF126256                        | <b>AAS66624.1</b>                                     |                  |          |
| ORF2                                      | <i>Haemophilus influenzae</i> A2              | n.d.     | M94855                          | <b>AAA24979.1</b>                                     |                  |          |
| HII699                                    | <i>Haemophilus influenzae</i> Rd              | n.d.     | U32842<br>NC_000907             | <b>AAC23345.1</b><br><b>NP_439841.1</b>               | Q48211           |          |
| $\alpha$ -2,3-sialyltransferase           | <i>Neisseria gonorrhoeae</i> F62              | 2.4.99.4 | U60664                          | <b>AAC44539.1</b><br>AAE67205.1                       | P72074           |          |
| $\alpha$ -2,3-sialyltransferase           | <i>Neisseria meningitidis</i> 126E, NRCC 4010 | 2.4.99.4 | U60662                          | <b>AAC44544.2</b>                                     |                  |          |
| $\alpha$ -2,3-sialyltransferase           | <i>Neisseria meningitidis</i> 406Y, NRCC 4030 | 2.4.99.4 | U60661                          | <b>AAC44543.1</b>                                     |                  |          |
| $\alpha$ -2,3-sialyltransferase (NMB0922) | <i>Neisseria meningitidis</i> MC58            | 2.4.99.4 | U60660<br>AE002443<br>NC_003112 | <b>AAC44541.1</b><br>AAF41330.1<br><b>NP_273962.1</b> | P72097           |          |
| NMA1118                                   | <i>Neisseria meningitidis</i> Z2491           | n.d.     | AL162755<br>NC_003116           | CAB84380.1<br><b>NP_283887.1</b>                      | <b>Q9JUV5</b>    |          |
| PM0508                                    | <i>Pasteurella multocida</i> PM70             | n.d.     | AE006086<br>NC_002663           | <b>AAK02592.1</b><br>NP_245445.1                      | Q9CNC4           |          |
| WaaH                                      | <i>Salmonella enterica</i> SARB25             | n.d.     | AF519787                        | <b>AAM82550.1</b>                                     | Q8KS93           |          |
| WaaH                                      | <i>Salmonella enterica</i> SARB3              | n.d.     | AF519788                        | <b>AAM82551.1</b>                                     | Q8KS92           |          |
| WaaH                                      | <i>Salmonella enterica</i> SARB39             | n.d.     | AF519789                        | <b>AAM82552.1</b>                                     |                  |          |
| WaaH                                      | <i>Salmonella enterica</i> SARB53             | n.d.     | AF519790                        | <b>AAM82553.1</b>                                     |                  |          |
| WaaH                                      | <i>Salmonella enterica</i> SARB57             | n.d.     | AF519791                        | <b>AAM82554.1</b>                                     | Q8KS91           |          |
| WaaH                                      | <i>Salmonella enterica</i> SARB71             | n.d.     | AF519793                        | <b>AAM82556.1</b>                                     | Q8KS89           |          |
| WaaH                                      | <i>Salmonella enterica</i>                    | n.d.     | AF519792                        | <b>AAM82555.1</b>                                     | Q8KS90           |          |

FIG. 2L

| Protein  | Organism  | EC#      | GenBank / GenPept     |                                  | SwissProt | PDB / 3D |
|--|---|----------|-----------------------|----------------------------------|-----------|----------|
|  | SARB8   |          |                       |                                  |           |          |
| WaaH   | <i>Salmonella enterica</i><br>SARC10V                       | n.d.     | AF519779              | <b>AAM88840.1</b>                | Q8KS99    |          |
| WaaH (fragment)  | <i>Salmonella enterica</i><br>SARC12                        | n.d.     | AF519781              | <b>AAM88842.1</b>                |           |          |
| WaaH (fragment)  | <i>Salmonella enterica</i><br>SARC13I                       | n.d.     | AF519782              | <b>AAM88843.1</b>                | Q8KS98    |          |
| WaaH (fragment)  | <i>Salmonella enterica</i><br>SARC14I                       | n.d.     | AF519783              | <b>AAM88844.1</b>                | Q8KS97    |          |
| WaaH   | <i>Salmonella enterica</i><br>SARC15II                      | n.d.     | AF519784              | <b>AAM88845.1</b>                | Q8KS96    |          |
| WaaH   | <i>Salmonella enterica</i><br>SARC16II                      | n.d.     | AF519785              | <b>AAM88846.1</b>                | Q8KS95    |          |
| WaaH (fragment)  | <i>Salmonella enterica</i><br>SARC3I                        | n.d.     | AF519772              | <b>AAM88834.1</b>                | Q8KSA4    |          |
| WaaH (fragment)  | <i>Salmonella enterica</i><br>SARC4I                        | n.d.     | AF519773              | <b>AAM88835.1</b>                | Q8KSA3    |          |
| WaaH   | <i>Salmonella enterica</i><br>SARC5II                       | n.d.     | AF519774              | <b>AAM88836.1</b>                |           |          |
| WaaH   | <i>Salmonella enterica</i><br>SARC6II                       | n.d.     | AF519775              | <b>AAM88837.1</b>                | Q8KSA2    |          |
| WaaH   | <i>Salmonella enterica</i><br>SARC8                         | n.d.     | AF519777              | <b>AAM88838.1</b>                | Q8KSA1    |          |
| WaaH   | <i>Salmonella enterica</i><br>SARC9V                        | n.d.     | AF519778              | <b>AAM88839.1</b>                | Q8KSA0    |          |
| UDP-glucose : $\alpha$ -1,2-glucosyltransferase (WaaH)     | <i>Salmonella enterica</i> subsp. <i>arizonae</i><br>SARC 5 | 2.4.1.-  | AF511116              | <b>AAM48166.1</b>                |           |          |
| bifunctional $\alpha$ -2,3/-2,8-sialyltransferase (Cst-II) | <i>Campylobacter jejuni</i> ATCC 43449                      | n.d.     | AF401529              | <b>AAL06004.1</b>                | Q93CZ5    |          |
| Cst  | <i>Campylobacter jejuni</i> 81-176                          | n.d.     | AF305571              | <b>AAL09368.1</b>                |           |          |
| $\alpha$ -2,3-sialyltransferase (Cst-III)                  | <i>Campylobacter jejuni</i> ATCC 43429                      | 2.4.99.- | AY044156              | <b>AAK73183.1</b>                |           |          |
| $\alpha$ -2,3-sialyltransferase (Cst-III)                  | <i>Campylobacter jejuni</i> ATCC 43430                      | 2.4.99.- | AF400047              | <b>AAK85419.1</b>                |           |          |
| $\alpha$ -2,3-sialyltransferase (Cst-II)                   | <i>Campylobacter jejuni</i> ATCC 43432                      | 2.4.99.- | AF215659              | <b>AAG43979.1</b>                | Q9F0M9    |          |
| $\alpha$ -2,3/8-sialyltransferase (CstII)                  | <i>Campylobacter jejuni</i> ATCC 43438                      | n.d.     | AF400048              | <b>AAK91725.1</b>                | Q93MQ0    |          |
| $\alpha$ -2,3-sialyltransferase cst-II                     | <i>Campylobacter jejuni</i> ATCC 43446                      | 2.4.99.- | AF167344              | <b>AAF34137.1</b>                |           |          |
| $\alpha$ -2,3-sialyltransferase (Cst-II)                   | <i>Campylobacter jejuni</i> ATCC 43456                      | 2.4.99.- | AF401528              | <b>AAL05990.1</b>                | Q93D05    |          |
| $\alpha$ -2,3/ $\alpha$ -2,8-sialyltransferase (CstII)     | <i>Campylobacter jejuni</i> ATCC 43460                      | 2.4.99.- | AY044868              | <b>AAK96001.1</b>                | Q938X6    |          |
| $\alpha$ -2,3/8-sialyltransferase (Cst-II)                 | <i>Campylobacter jejuni</i> ATCC 700297                     | n.d.     | AF216647              | <b>AAL36462.1</b>                |           |          |
| ORF  | <i>Campylobacter jejuni</i> GB11                            | n.d.     | AY422197              | <b>AAR82875.1</b>                |           |          |
| $\alpha$ -2,3-sialyltransferase cstIII                     | <i>Campylobacter jejuni</i> MSC57360                        | 2.4.99.- | AF195055              | <b>AAG29922.1</b>                |           |          |
| $\alpha$ -2,3-sialyltransferase cstIII Cj1140              | <i>Campylobacter jejuni</i> NCTC 11168                      | 2.4.99.- | AL139077<br>NC_002163 | <b>CAB73395.1</b><br>NP_282288.1 | Q9PNF4    |          |
| $\alpha$ -2,3/ $\alpha$ -2,8-sialyltransferase II (cstII)  | <i>Campylobacter jejuni</i> O:10                            | n.d.     | -<br>AX934427         | AAO96669.1<br><b>CAF04167.1</b>  |           |          |
| $\alpha$ -2,3/ $\alpha$ -2,8-sialyltransferase II (CstII)  | <i>Campylobacter jejuni</i> O:19                            | n.d.     | AX934431              | <b>CAF04169.1</b>                |           |          |
| $\alpha$ -2,3/ $\alpha$ -2,8-sialyltransferase II (CstII)  | <i>Campylobacter jejuni</i> O:36                            | n.d.     | AX934436              | <b>CAF04171.1</b>                |           |          |
| $\alpha$ -2,3/ $\alpha$ -2,8-                              | <i>Campylobacter</i>  | n.d.     | AX934434              | <b>CAF04170.1</b>                |           |          |

FIG. 2M

| Protein  | Organism                           | EC#      | GenBank / GenPept             |   | SwissProt    | PDB / 3D |
|--|------------------------------------|----------|-------------------------------|---|--------------|----------|
| sialyltransferase II (CstII)   | <i>jejuni O:4</i>                  |          |                               |   |              |          |
| $\alpha$ -2,3/ $\alpha$ -2,8-sialyltransferase II (CstII)                    | <i>Campylobacter jejuni O:41</i>   | n.d.     | -<br>AX934429                 | AAO96670.1<br>AAT17967.1<br>CAF04168.1  |              |          |
| $\alpha$ -2,3-sialyltransferase cst-I  | <i>Campylobacter jejuni OH4384</i> | 2.4.99.- | AF130466<br>-                 | AAF13495.1<br>AAS36261.1                | Q9RGF1       |          |
| bifunctional $\alpha$ -2,3-/2,8-sialyltransferase (Cst-II) HI0352 (fragment) | <i>Campylobacter jejuni OH4384</i> | 2.4.99.- | AF130984<br>AX934425          | AAF31771.1<br>CAF04166.1                | 1R07<br>1R08 | C<br>A   |
| PM1174   | <i>Pasteurella multocida PM70</i>  | n.d.     | U32720<br>X57315<br>NC_000907 | AAC22013.1<br>CAA40567.1<br>NP_438516.1 | P24324       |          |
| Sequence 10 from patent US 6503744   | Unknown.                           | n.d.     | AE006157<br>NC_002663         | AAK03258.1<br>NP_246111.1               | Q9CLP3       |          |
| Sequence 10 from patent US 6699705   | Unknown.                           | n.d.     | -                             | AAT17969.1                              |              |          |
| Sequence 12 from patent US 6699705   | Unknown.                           | n.d.     | -                             | AAT17970.1                              |              |          |
| Sequence 2 from patent US 6709834  | Unknown.                           | n.d.     | -                             | AAT23232.1                              |              |          |
| Sequence 3 from patent US 6503744  | Unknown.                           | n.d.     | -                             | AAO96668.1                              |              |          |
| Sequence 3 from patent US 6699705  | Unknown.                           | n.d.     | -                             | AAT17965.1                              |              |          |
| Sequence 34 from patent US 6503744   | Unknown.                           | n.d.     | -                             | AAO96684.1                              |              |          |
| Sequence 35 from patent US 6503744 (fragment)                                | Unknown.                           | n.d.     | -<br>-                        | AAO96685.1<br>AAS36262.1                |              |          |
| Sequence 48 from patent US 6699705   | Unknown.                           | n.d.     | -                             | AAT17988.1                              |              |          |
| Sequence 5 from patent US 6699705  | Unknown.                           | n.d.     | -                             | AAT17966.1                              |              |          |
| Sequence 9 from patent US 6503744  | Unknown.                           | n.d.     | -                             | AAO96671.1                              |              |          |

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US06/00282

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC: A61K 38/00( 2006.01)

USPC: 514/8  
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
U.S. : 514/8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
STN, EAST, Pubmed, Previous Related US Cases

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|------------|---|-----------------------|
| A          | Ito, T et al., Synthesis of Bioactive Sialosides, Pure & Appl. Chem ; 1993, Vol. 65, No. 4, pages 753-762, see entire document. | 1-18                  |
| A          | US 5,405,753 (Brossmer et al) 11 April 1995 (04.11.1995), see entire document.  | 1-18                  |

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| Date of the actual completion of the international search<br>30 April 2006 (30.04.2006)   | Date of mailing of the international search report<br><b>23 MAY 2006</b>                                  |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US<br>Commissioner for Patents<br>P.O. Box 1450<br>Alexandria, Virginia 22313-1450<br>Facsimile No. (571) 273-3201 | Authorized officer<br>Thomas S. Heard<br>Telephone No. (571) 272-1600<br><i>Janice Ford</i><br><i>jfd</i> |